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16. ABSTRACT

This study was conducted to determine the comparative effectiveness of chlorine, bromine chloride, and ozone as wastewater disinfectants, and to determine any residual toxicity associated with wastewater disinfection with these agents or with chlorinated wastewater which had been dechlorinated with sulfur dioxide.

A stream of nondisinfected trickling filter effluent was pumped from the Wyoming, Michigan Wastewater Treatment Plant to the project's water treatment building. The supply of effluent was split into four streams, three of which were disinfected with either chlorine, bromine chloride, or ozone and then delivered to the bioassay laboratory for residual toxicity tests. The fourth stream was delivered directly to the bioassay laboratory for testing. In addition, a portion of the chlorinated effluent stream was dechlorinated with sulfur dioxide and then pumped to the bioassay laboratory.

Total and fecal coliform densities, total suspended solids, volatile solids, COD, ammonia nitrogen, phosphate, turbidity, color, and pH were measured in the wastewater streams. Each of the five wastewater streams was tested for acute toxicity towards several species of fishes and invertebrates, and chronic toxicity in a life cycle study with the fathead minnow, Pimephales promelas, as the test subject.

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DISINFECTION EFFICIENCY AND RESIDUAL TOXICITY OF SEVERAL WASTEWATER DISINFECTANTS

Volume II - Wyoming, Michigan

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment,and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This study was concerned with comparing the disinfection efficiency of chlorine with and without dechlorination, ozone, and bromine chloride on parallel wastewater streams and evaluating the potential toxicity of those streams to aquatic life. Intimate knowledge of wastewater disinfection principles and the effects of wastewater disinfection practices on man and his environment is vital to the proper control of disease transmission and preservation of wildlife. This project has contributed valuable information in the quest for these goals.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

This study was conducted to determine the comparative effectiveness of chlorine, bromine chloride, and ozone as wastewater disinfectants, and to determine any residual toxicity associated with wastewater disinfection with these agents or with chlorinated wastewater which had been dechlorinated with sulfur dioxide.

A stream of nondisinfected trickling filter effluent was pumped from the Wyoming, Michigan Wastewater Treatment Plant to the project's water treatment building. The supply of effluent was split into four streams, three of which were disinfected with either chlorine, bromine chloride, or ozone and then delivered to the bioassay laboratory for residual toxicity tests. The fourth stream was delivered directly to the bioassay laboratory for testing. In addition, a portion of the chlorinated effluent stream was dechlorinated with sulfur dioxide and then pumped to the bioassay laboratory.

Total and fecal coliform densities, total suspended solids, volatile solids, COD, ammonia nitrogen, phosphate, turbidity, color, and pH were measured in the wastewater streams. Each of the five wastewater streams was tested for acute toxicity towards several species of fishes and invertebrates, and chronic toxicity in a life cycle study with the fathead minnow, Pimephales promelas, as the test subject.

Ozone was found to be nontoxic to fish at the concentrations normally expected to exist in receiving waters from plants practicing ozone disinfection. Sulfur dioxide eliminated the inherent toxicity of chlorine, and bromine chloride was less toxic than chlorine.

The disinfection effectiveness of ozone was severely impaired by the lack of a reliable method of pacing dosage with shifts in demand. No problems were experienced with the chlorination or sulfonation equipment. Disinfection by bromine chloride was impaired by occasional malfunctions in the dosing equipment.

This report was submitted in fulfillment of Grant No. S-802292 by the City of Wyoming, Michigan under the partial sponsorship of the U.S. Environmental Protection Agency. Investigative research was performed jointly under subcontract by the City of Wyoming, Michigan and Grand Valley State Colleges. This report covers the period from July 1975 to June 1976.

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SECTION I

INTRODUCTION

AN OVERVIEW OF THE PROJECT

This report deals with the second half of a two-part project designed to investigate the bactericidal efficacy of chlorine and alternative disinfectants in parallel on identical wastewater streams, and evaluate any undesirable residual toxic effects associated with those alternative processes. The reader is referred to the INTRODUCTION of Volume I of this report for an overview of the problem and related research.

This project was conducted at two study sites, the Grandville, Michigan, Wastewater Treatment Plant and the Wyoming, Michigan, Wastewater Treatment Plant. The Grandville plant is an activated sludge facility that treats wastewater derived almost totally from domestic sources. The Wyoming plant has both activated sludge and trickling filter facilities and treats an influent composed of 35-45 percent industrial wastes. This study utilized trickling filter effluent from the Wyoming plant.

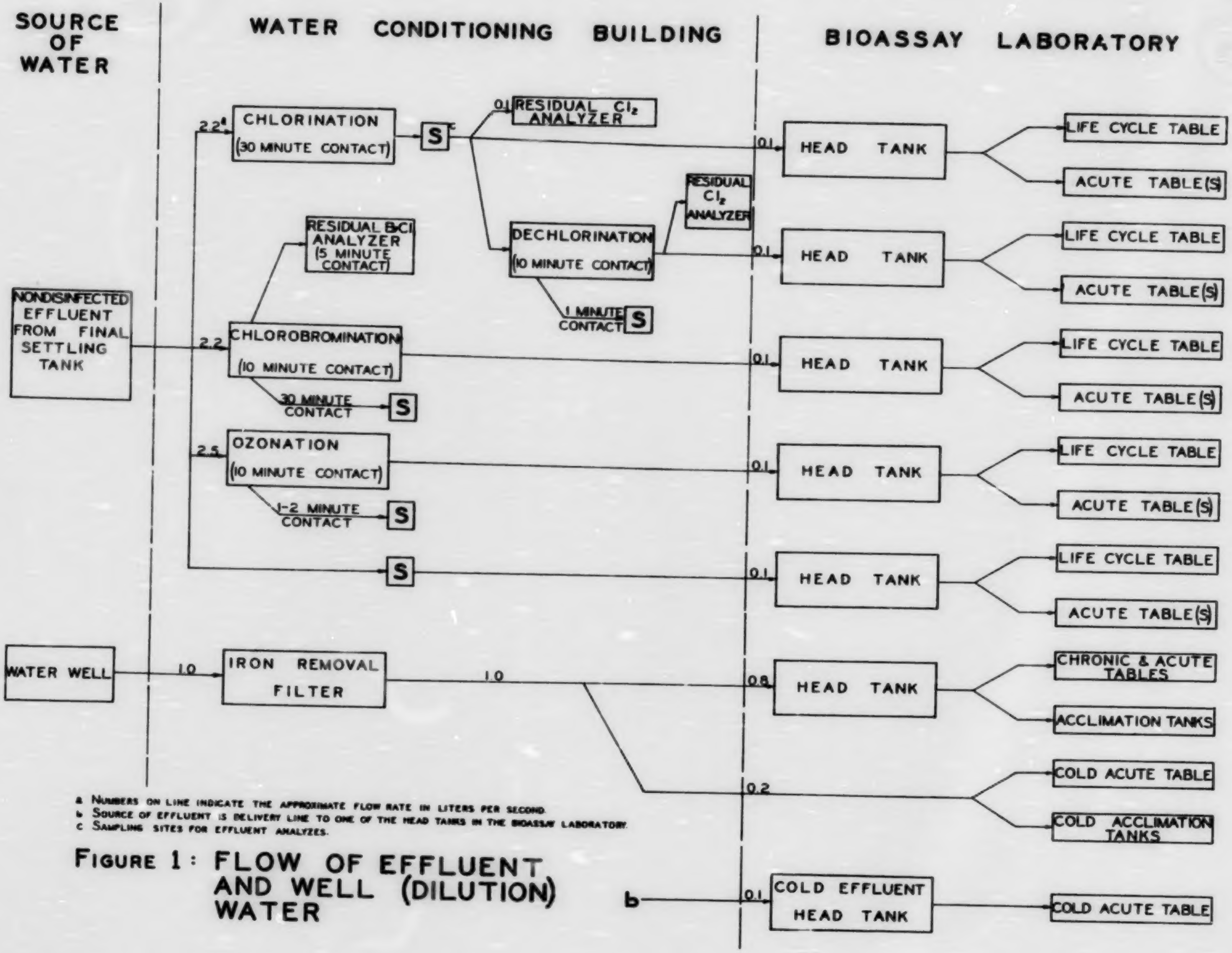
The specific objectives of this study were to evaluate simultaneously the efficacy of chlorine (Cl_2) with and without sulfur dioxide (SO_2) dechlorination, ozone (O_3), and bromine chloride (BrCl) to disinfect identical streams of a high industrial waste content wastewater that had been treated by the trickling filter process, and to determine the extent to which the treated effluents were toxic to aquatic life.

THE WYOMING STUDY SITE

The Wyoming Wastewater Treatment Plant includes both activated sludge and trickling filter treatment facilities which may be operated in series or in parallel. The design capacity of the plant is 72,000 cu m/d (19 mgd) and the average inflow during the study period was 44,000 cu m/d (11.6 mgd).

Wastewater flow to the trickling filter averaged approximately 5700 cu m/d (1.5 mgd). After final settling, nondisinfected effluent was pumped to the water treatment building for disinfection with Cl_2, BrCl, or O_3 (Figure 1). In addition, a portion of the chlorinated stream was dechlorinated. Detailed information on the flow rates, dose rates and resulting residual, and other characteristics of the wastewater are presented in Section III of this report, while bacteriological data are presented in Section IV.

The various effluent streams were pumped from the water treatment building


SOURCE OF WATER
WATER CONDITIONING BUILDING
BIOASSAY LABORATORY
NONDISINFECTED EFFLUENT FROM FINAL SETTLING TANK
WATER WELL
2.2a
CHLORINATION (30 MINUTE CONTACT)
S c
0.1
RESIDUAL Cl_2 ANALYZER
DECHLORINATION (10 MINUTE CONTACT)
RESIDUAL Cl_2 ANALYZER
1 MINUTE CONTACT
S
RESIDUAL BrCl ANALYZER (5 MINUTE CONTACT)
2.2
CHLOROBROMINATION (10 MINUTE CONTACT)
30 MINUTE CONTACT
S
2.5
OZONATION (10 MINUTE CONTACT)
1-2 MINUTE CONTACT
S
S
1.0
IRON REMOVAL FILTER
1.0
0.1
0.1
0.1
0.1
0.1
0.8
0.2
0.1
HEAD TANK
HEAD TANK
HEAD TANK
HEAD TANK
HEAD TANK
HEAD TANK
b
COLD EFFLUENT HEAD TANK
LIFE CYCLE TABLE
ACUTE TABLE(S)
LIFE CYCLE TABLE
ACUTE TABLE(S)
LIFE CYCLE TABLE
ACUTE TABLE(S)
LIFE CYCLE TABLE
ACUTE TABLE(S)
LIFE CYCLE TABLE
ACUTE TABLE(S)
CHRONIC & ACUTE TABLES
ACCLIMATION TANKS
COLD ACUTE TABLE
COLD ACCLIMATION TANKS
COLD ACUTE TABLE

[a] Numbers on line indicate the approximate flow rate in liters per second.
[b] Source of effluent is delivery line to one of the head tanks in the bioassay laboratory.
[c] Sampling sites for effluent analyzes.

FIGURE 1: FLOW OF EFFLUENT AND WELL (DILUTION) WATER

to their respective head tanks in the bioassay laboratory which was located in an adjacent building (Figure 1). Well water was passed through an iron removal filter in the water treatment building and delivered to a head tank in the bioassay laboratory where it served as a dilution water supply. The various water supplies were adjusted to test temperature in their head tanks and, after a detention time of 30-45 minutes, were then delivered by gravity flow to proportional diluters on the acute and life cycle test tables. Dilution water and seven concentrations of each effluent type were delivered to aquaria in which test subjects were maintained. Several species of fishes and invertebrates served as subjects for acute toxicity studies, while fathead minnows (Pimephales promelas) were the subjects in the life cycle studies. Bioassay results are presented in Sections V and VI of this report.

SECTION II

CONCLUSIONS

1. With the exception of the residual toxicity to aquatic organisms imparted to the effluent by some of the disinfection processes, no change in wastewater quality that might create an environmental problem under the typical conditions of effluent release to waterways was observed as the result of disinfection with chlorine, bromine chloride, or ozone, or dechlorination with sulfur dioxide.

2. All of the disinfectants tested had the capability to reduce total and fecal coliform densities to project standards.

3. While the efficiency of each of the disinfectants was directly related to effluent quality, ozone was most dependent and chlorine least dependent upon this factor.

4. Malfunctions of the bromine chloride and ozone dosing systems demonstrated the need for technological improvement in these systems.

5. Undiluted nondisinfected trickling filter effluent from the Wyoming Wastewater Treatment Plant was lethal to young fathead minnows (*Pimephales promelas*).

6. Any adverse affects on the growth or reproduction of fathead minnows were not attributed to any of the disinfectants tested.

7. Chlorine was the only disinfectant tested which produced a lethal residual toxicity effect in life cycle studies with fathead minnows.

8. Life cycle studies showed that fathead minnows were more tolerant of residual chlorine in Wyoming effluent, which contained 35-45 percent industrial wastes, than in Grandville effluent, which was primarily of domestic origin. For example, the probability of test animals surviving through 60 days of age was similar in mean residual chlorine concentrations of 0.076 mg/l in a 20 percent concentration of Grandville effluent and 0.161 mg/l in a 25 percent concentration of Wyoming effluent.

9. Dechlorination with sulfur dioxide eliminated the lethal effects of chlorinated effluent and produced no adverse effects on the survival, growth or reproduction of fathead minnows.

10. The only treatment-related mortality of fathead minnows in the life cycle study with chlorobrominated effluent resulted from an apparent synergistic effect between residual bromine chloride and some constituent of the wastewater.

11. Fathead minnow survival was not adversely affected in the life cycle study with ozonated effluent.

12. Chlorine was generally the most acutely toxic disinfectant tested as evidenced by its consistency in producing mortality in acute toxicity tests. However, salmonid fishes and the pugnose shiner (*Notropis anagenus*) were more tolerant of higher concentrations of residual chlorine than of residual bromine chloride.

SECTION III

THE WASTEWATER TREATMENT SYSTEMS AND THE CHARACTERISTICS OF THE WASTEWATER STREAMS

INTRODUCTION

A variety of interactions and effects have been shown to occur when disinfectants are applied to wastewater. For instance, the organic load and the pH of the wastewater often affect the bactericidal activity of a disinfectant. Conversely, the disinfectant may directly affect one or more of the wastewater properties, such as dissolved oxygen or pH, or may render the effluent toxic to aquatic organisms.

This section discusses the characteristics of the Wyoming wastewater and the design of the test treatment systems and their effects on the characteristics of the wastewater.

THE WYOMING WASTEWATER TREATMENT PLANT

The wastewater treatment plant at Wyoming, Michigan includes both trickling filter and activated sludge systems, which may be operated singly, in series or in parallel. The influent wastewater is of mixed origin, with approximately 55-65 percent being from domestic sources and 35-45 percent from light industry, metal plating plants, a dairy products processor, and commercial establishments. The plant was recently expanded to a capacity of 72,000 cu m/d (19 mgd) and the operational and equipment problems typically associated with the new plant start-ups resulted in a highly variable effluent quality during this study. The accumulation of sludge in the treatment systems during the December-May period was especially troublesome and resulted in reduced effluent quality from both the trickling filter and activated sludge treatment systems.

The average plant inflow during the study period was 44,000 cu m/d (11.6 mgd) with a mean biochemical oxygen demand of 321 mg/l. About 5,700 cu m/d (1.5 mgd) of this influent was passed through the trickling filter treatment system and served as the source of the effluent for this study. The characteristics of the raw wastewater and test streams are summarized in Table 1.

Figure 2 illustrates the monthly and study period averages of total suspended solids in the nondisinfected trickling filter effluent. Monthly mean total suspended solids levels in the raw wastewater ranged from 204-422 mg/l ($\bar{x}$= 293 mg/l) and generally tended to increase during the study period. The average removal of total suspended solids by the trickling filter was 74 percent.

Table 1. MEAN CHARACTERISTICS OF THE INFLUENT AND THE TEST STREAMS[a] DURING THE STUDY PERIOD JUNE 16, 1975, THROUGH APRIL 30, 1976

Parameter	Plant Influent	Test Stream					
		Nondis-infected	Chlor-inated	Dechlor-inated	Chloro-brominated	Filtered	Filtered & Ozonated
Total Suspended Solids (mg/1)	293	75 (57)[b]	59 (50)	61 (49)	58 (48)	19 (16)	21 (17)
Volatile Suspended Solids (mg/1)	212	55 (43)	43 (35)	44 (34)	43 (37)	15 (14)	16 (15)
Turbidity (J.T.U.)	N.A.[c]	97 (71)	71 (45)	70 (46)	71 (47)	48 (42)	29 (31)
Apparent Color (Platinum Cobalt units)	N.A.	275 (187)	206 (117)	207 (122)	205 (123)	146 (103)	85 (75)
True Color (Platinum Cobalt units)	N.A.	67 (26)	57 (18)	55 (17)	57 (20)	86 (51)	36 (32)
Chemical Oxygen Demand (mg/1)	N.A.	146 (89)	136 (80)	141 (79)	135 (79)	97 (60)	94 (59)
Ammonia Nitrogen (mg/1)	14.8	9.3 (1.9)	9.1 (1.9)	9.2 (1.9)	8.9 (1.9)	9.3 (2.0)	9.3 (1.8)
Total Phosphorus (mg/1)	7.5	6.2 (2.4)	6.0 (2.2)	5.8 (1.5)	5.9 (2.2)	5.0 (1.8)	5.1 (2.2)
Dissolved Oxygen (mg/1)	N.A.	1.1 (0.9)	1.9 (1.2)	3.4 (1.1)	1.8 (0.9)	0.5 (0.9)	9.0 (1.7)

[a] The characteristics of the disinfected streams were measured at the sites shown in Figure 1.

[b] Numbers in parentheses are standard deviations

[c] Data not available

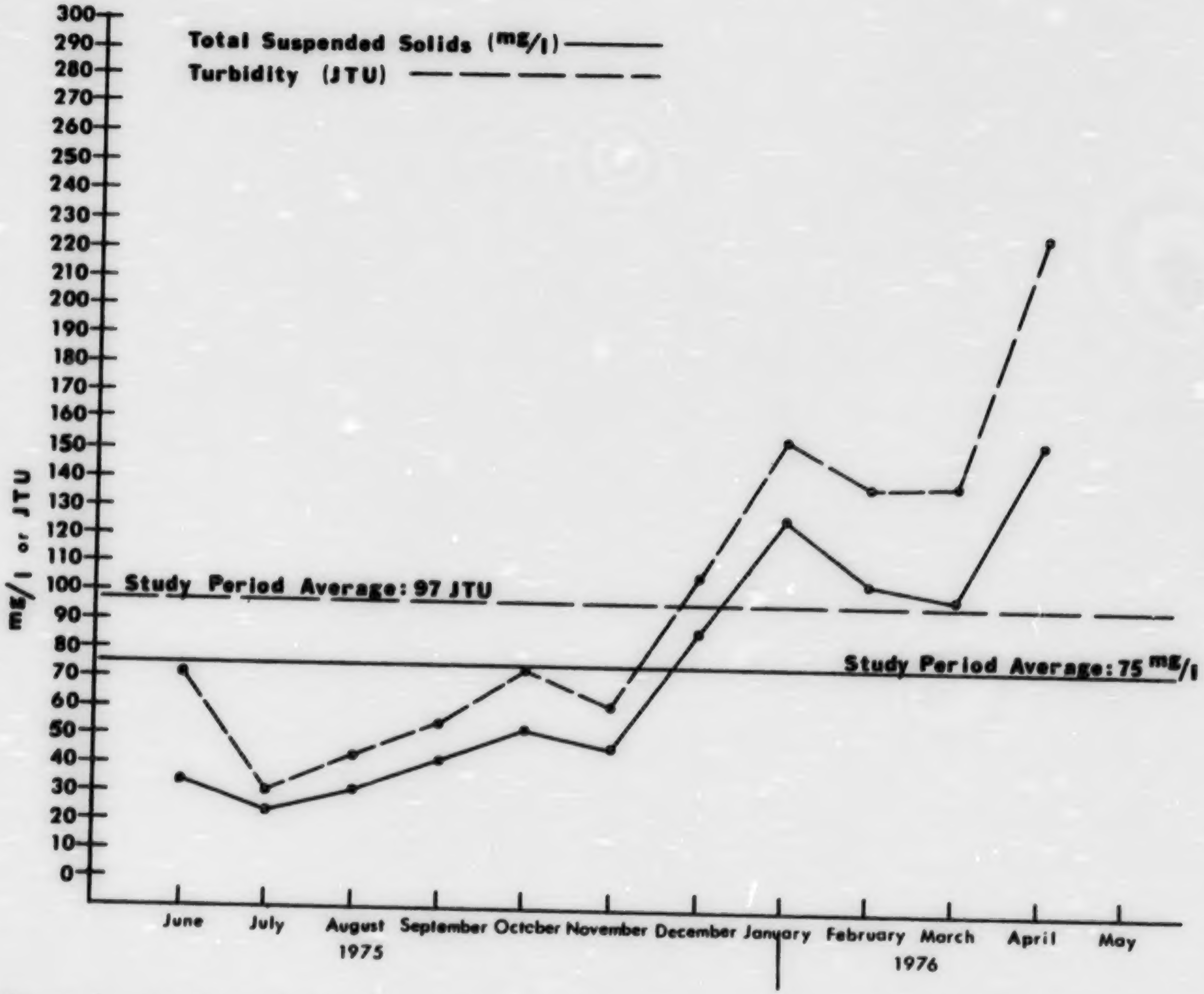


Figure 2. Study period and monthly average turbidity and total suspended solids levels in the nondisinfected trickling filter effluent.

The chemical oxygen demand of the nondisinfected effluent averaged 146 mg/l (Table 1 and Figure 3). The mean effluent total phosphate and ammonia nitrogen concentrations were 6.2 and 9.3 mg/l, respectively (Figure 4). The trickling filter averaged 17 percent removal of phosphate and 37 percent removal of ammonia nitrogen.

Figures 2-4 illustrate the general decline in effluent quality during the course of the study. Other effluent parameters exhibited a similar trend. This change in quality caused a significant increase in disinfectant demand in the effluent, thereby moderately affecting disinfection efficiency and residual disinfectant concentrations.

DESIGN OF TEST WASTEWATER TREATMENT SYSTEMS

A water well was drilled adjacent to the water treatment building to provide chlorine-free dilution water for the bioassay laboratory. After passage through an iron removal filter in the water treatment building, well water was pumped to a head tank in the bioassay laboratory (Figure 1).

After final settling, nondisinfected effluent was pumped to the water treatment building and divided into four streams: one was treated with Cl_2, one with BrCl, one with O_3, and one was passed directly to the bioassay laboratory (Figure 1). A portion of the chlorinated effluent from the end of the chlorine contact chamber was dechlorinated with SO_2. All five streams were then pumped to respective head tanks in the bioassay laboratory.

Chlorine was injected into the wastewater by an aspirator, using a Wallace and Tiernan Model 20-055 chlorinator. The chlorinated effluent flowed into a steel contact chamber having a 30-minute residence time at a flow rate of 2.2 l/sec (35 gpm). The steel contact chamber was 3.7 m long by 1.2 m wide by 0.9 m deep with steel baffles welded to the bottom and sides at intervals of 1.2 and 2.4 m from the end, and wooden baffles inserted from the top at 0.6, 1.8, and 3.0 m from the end. This arrangement of baffles provided an under-over-under flow configuration. A Fisher-Porter Anachlor continuous titrator was installed in the line at the end of the Cl_2 contact chamber to continuously monitor the 30 minute residual Cl_2 concentration. The readouts were used as the basis for the manual adjustment of Cl_2 metering rates so that a constant Cl_2 residual could be maintained. The Cl_2 residual was normally checked at 1-2 hour intervals, and metering adjustments were made as necessary.

The 30 minute residual Cl_2 concentration was maintained at 2.0 mg/l throughout the study. The average Cl_2 dosage required to maintain this residual was 5.9 mg/l.

A portion of the chlorinated stream (1.0 l/sec (15 gpm)) was treated with SO_2. The SO_2 was injected into the chlorinated effluent by an aspirator and regulated by a Wallace and Tiernan Model 20-055 chlorinator. The dechlorinated stream flowed into a fiberglass contact chamber having a residence time of 10 minutes at a flow rate of 1.0 l/sec. The contact chamber was 1.8 m long by 0.6 m wide by 0.6 m deep and contained three baffles to produce an under-over-under flow configuration.

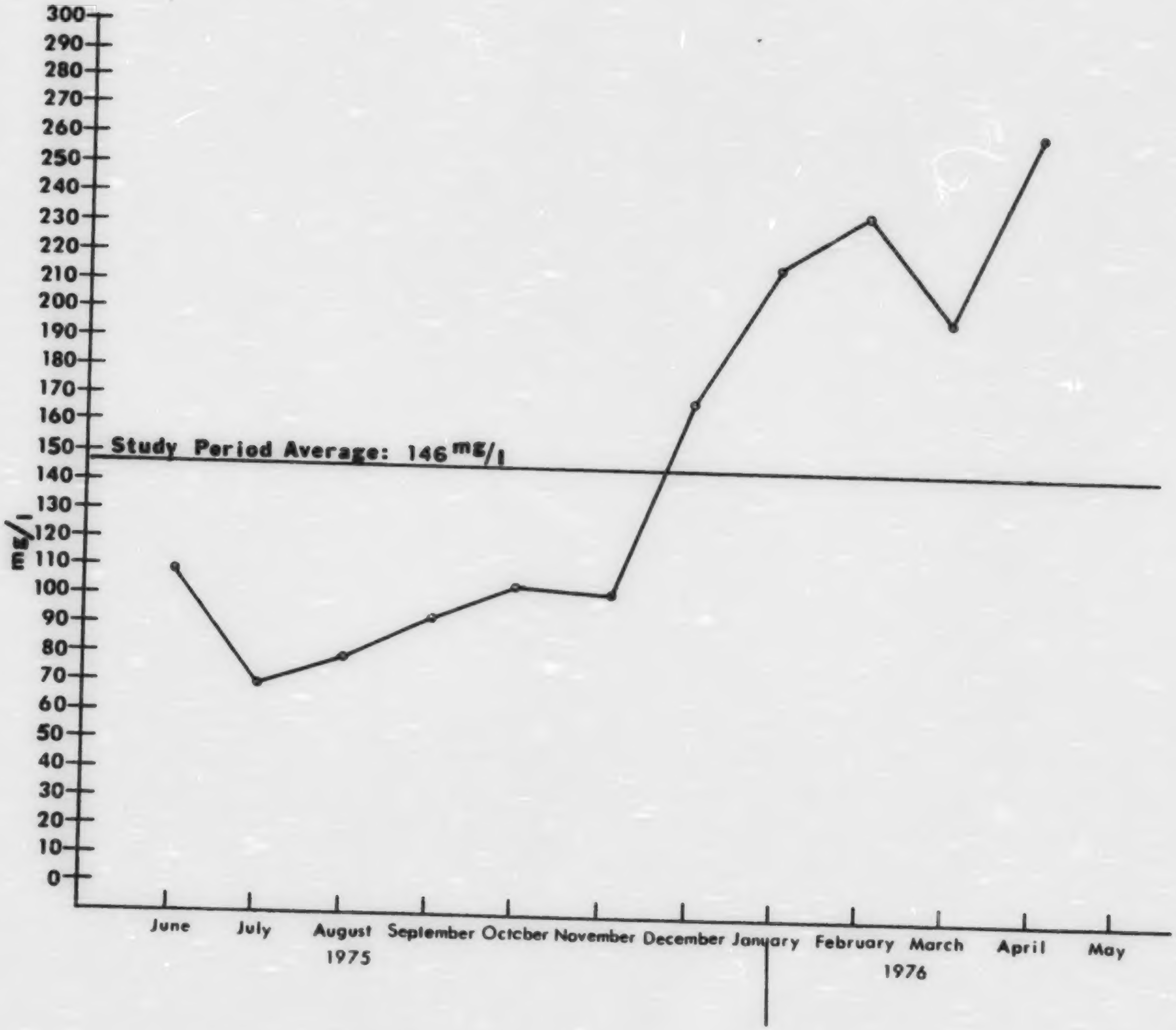


Figure 3. Study period and monthly mean COD values.

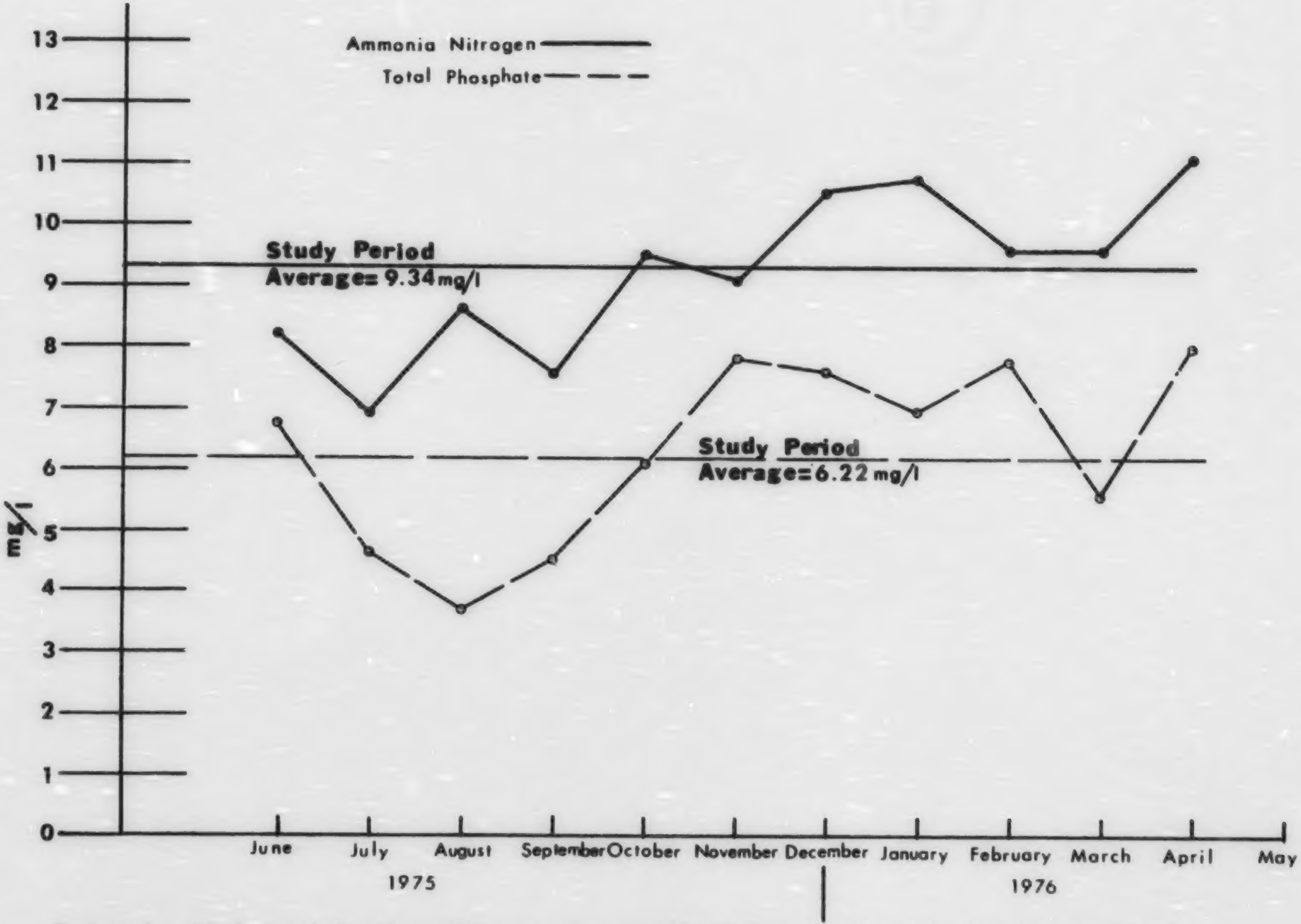


Figure 4. Study period and monthly average ammonia nitrogen and total phosphate values.

A Fisher-Porter Anachlor continuous titrator unit received flow of dechlorinated effluent from the end of the SO_2 contact chamber. This analyzer was modified to sound an alarm and shut off the dechlorinated effluent supply to the bioassay test chambers in the event that residual Cl_2 was present in the dechlorinated effluent.

To further protect the bioassay test animals from accidental breakthrough of Cl_2 in the dechlorinated stream, the residual sulfite concentration was maintained at approximately 5 mg/l.

Prior to February, liquid BrCl was vaporized by heat and fed to the effluent by a Wallace and Tiernan Model 20-055 chlorinator. From February until the end of the study, BrCl was fed by a Capitol Controls prototype BrCl feeder which vaporized the liquid BrCl in a constant temperature warm water bath. The BrCl contact chamber was identical to the Cl_2 contact chamber. Chlorobrominated effluent was delivered to the bioassay laboratory after a 10 minute residence in the contactor.

A Fisher-Porter Anachlor continuous titrator was installed to measure residual BrCl after a five minute residence time. Readings were made and dosages were adjusted on the same schedule as the chlorination system. The five minute BrCl residual was maintained at 0.4-0.5 mg/l by feeding an average of 4.2 mg/l.

The ozonation system consisted of an Ingersoll-Rand Model ESV-NL air compressor, a Pall-Trinity Model 35 HA1 dryer, a modified W. R. Grace Model LG-16 ozonator, a W. R. Grace positive pressure injection contacting system, and a contact chamber. A Wellsbach Model CL-34-D19L ozone generator served as a standby ozone source. Air from the compressor was dried to a -50C dew point prior to delivery to the O_3 generator. Prior to O_3 injection the effluent was filtered through a Baker Model HRC Hi-Rate filter system to remove suspended solids. The effluent was then mixed with the ozonated air at the top of a 3.7 m column through the positive pressure injector. The gas/liquid mixture flowed cocurrently from the injector down a 10.2 cm diameter central pipe. The mixture reversed direction at the base of the central pipe, flowed upwards through a concentric 20 cm diameter circular tank, down again through a concentric 30.5 cm circular tank, and emerged from the bottom through a hose to a covered steel contact chamber (1.2 m long x 1.2 m wide x 0.9 m deep). Detention time was approximately 40 seconds in the vertical column and 10 minutes in the steel contact chamber. About 28 percent of the ozone that was introduced was lost in the off-gas from the contactor.

Ozone dosages ranged from 4.5 to 12 mg/l, calculated on the basis of the amount of ozone in the gas stream minus that lost in the off-gas in relation to the liquid flow to the contactor.

The sampling sites for chemical analyses of the various effluent streams are shown in Figure 1. Identical samples were taken from each stream during the period June 9, 1975, to April 30, 1976, five days per week, usually between 2 and 4 P.M. Since residence times varied in the different contactor systems, designated sample sites may have varied by as much as 30 minutes. However, such short term variation was probably of little significance compared to the longer term variations in the effluent.

REACTIONS OF DISINFECTANTS

See pages 19-23 in Volume I of this report[1].

MATERIALS AND METHODS

The materials and methods for chemical tests on the effluent streams were identical to those described on pages 23 and 24 in Volume I of this report, with the exception that residual Cl_2 and BrCl were measured by the modified amperometric titration method described on page 60 of Volume I. Statistical differences among respective mean test results were determined by subjecting the data to a two-tailed t-test.

RESULTS AND DISCUSSION

Mean total and volatile suspended solids levels in all of the treated test streams (Table 1) were significantly lower ($P < 0.01$) than in the nondisinfected effluent stream. The sedimentation which occurred in the contact chambers and the filtration process accounted for most of the observed decrease in suspended solids. The small differences between the mean total and volatile suspended solids levels in filtered effluent and ozonated filtered effluent were not statistically significant.

The mean turbidity of each treated stream was significantly lower than that of the nondisinfected effluent (Table 1). Again, the settling time in the various contact chambers probably accounted for most of the observed decreases in turbidity.

Mean apparent and true color levels in all of the treated effluent streams were significantly ($P < 0.001$) lower than those of the nondisinfected effluent. Filtration had a significant ($P < 0.001$) effect on mean true and apparent color levels when contrasted with the respective means for the nondisinfected stream. Likewise, the mean apparent and true color levels in ozonated effluent were significantly different ($P < 0.001$) from the respective means for filtered effluent. Thus, in view of the varied contact and settling times in the different treatment systems, ozonation appeared to have the greatest potential of the disinfectants tested to reduce color levels, especially the true color of the effluent. Other investigators[3-5] have similarly found ozone to be a good decolorizer.

None of the disinfection processes produced a significant change in mean chemical oxygen demand (COD) levels. This contrasts with reports[2,3,6,7] of COD reductions after ozonation. Filtration produced a significant ($P < 0.001$) reduction in the mean COD of the nondisinfected effluent.

When contrasted with nondisinfected effluent, only the filtered test stream exhibited a significant ($P < 0.001$) reduction in mean total phosphate concentration.

The mean dissolved oxygen (DO) levels in the chlorinated and chlorobrominated effluent streams were not significantly different from the mean observed for nondisinfected effluent. However, the dechlorinated effluent had a significantly higher mean DO ($P < 0.001$) concentration than the nondisinfected effluent, primarily because of the aeration which occurred as chlorinated effluent was pumped to the dechlorination treatment system. While the DO in the filtered effluent was significantly ($P < 0.001$) lower than the DO in the nondisinfected effluent, ozonation significantly ($P < 0.001$) increased the mean DO of the filtered stream, as expected.

The pH of the effluent was not significantly altered by any of the disinfection systems.

The most notable difference between these data and those from the study conducted at Grandville ((Volume I of this report) was the generally poorer quality of the Wyoming effluent as evidenced by all of the wastewater characteristics presented in Table 1. This lower effluent quality had the potential to affect the results of the bacteriological and bioassay tests.

CONCLUSIONS

The quality of the effluent tested in this study was substantially lower than the quality of the effluent tested in the Grandville study.

The physical and chemical characteristics of the Wyoming effluent test streams were affected by necessary differences in the dosing and contact systems of the various disinfectants. Nevertheless, none of the test treatments produced adverse changes in the water quality parameters measured, all treatments reduced effluent color, and ozonation improved effluent DO concentrations.

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SECTION IV

DISINFECTION STUDIES

INTRODUCTION

The role of disinfection in wastewater treatment is to destroy pathogenic microorganisms in the wastewater and thereby provide a reasonable margin of safety in controlling the spread of disease in natural waters. Total and fecal coliform concentrations have been widely used as fecal pollution indicators, and maximum allowable concentrations of these bacteria have been assigned as public health standards.[1]

The objective of this part of the project was to evaluate the efficiency of wastewater disinfection by chlorination (with and without dechlorination), chlorobromination, and ozonation on parallel wastewater effluent streams. The experimental site was the Wyoming, Michigan wastewater treatment plant, which receives both industrial and domestic wastewater. For a description of the Wyoming study site and a detailed discussion of the effectiveness and chemical reactivity of the disinfection processes, refer to Section III.

MATERIALS AND METHODS

The five tube-three dilution multiple tube fermentation method (MPN)[1] was used to enumerate coliform bacteria in the five effluent streams. For determining total coliform densities, Lauryl Tryptose broth (Difco) was used as the presumptive medium[1] and Brilliant Green Bile broth (Difco) as the confirmatory medium. EC broth was used for fecal coliform determinations. Grab samples of nondisinfected, chlorinated, chlorobrominated, and ozonated effluent were collected daily in sterile bottles containing sodium thiosulfate.[1]

A number of bacteriological measurements were performed to determine if significant diurnal fluctuations in coliform numbers occurred in the Wyoming effluent. When it was found that coliform levels were fairly constant throughout the day, the sampling time was set for mid-afternoon to accommodate two working shifts. Based on findings from the Grandville study, samples were collected at the following locations:

(1) Nondisinfected wastewater - immediately prior to entering the treatment systems.

(2) Chlorinated effluent - at a site in the chlorine contact chamber corresponding to a detention time of 30 minutes (see figure 1).

(3) Dechlorinated effluent - at a site in the dosing unit immediately following the SO_2 injection point.

(4) Chlorobrominated effluent - at the end of the BrCl contact chamber, corresponding to a mean detention time of 30 minutes.

(5) Ozonated effluent - from a sample tap immediately following the contact unit, corresponding to a detention time of 1 to 2 minutes.

Data were tabulated and statistically analyzed to evaluate the pertinent relationships among the various wastewater characteristics.

The target coliform densities were arbitrarily established at 200 fecal coliforms per 100 ml and 1000 total coliforms per 100 ml. Disinfection efficiency was calculated by dividing the number of bacteriological samples which met the above standards by the total number of samples taken from each effluent.

Chlorine and bromine chloride residuals and ozone dosages were measured as described in Section III. Halogen dosages were determined by cylinder weight-loss and rotameter flow rates.

All data were subjected to statistical analyses. Significant differences in geometric mean coliform densities among the various disinfection systems were determined by t-test, analysis of variance, and least significant difference (a priori).

RESULTS

Observations of Accumulated Data

In order to determine and compare the relative bactericidal effects of each disinfectant under similar conditions, coliform data were grouped into two time segments during which wastewater quality was fairly consistent. The first segment, from June through December, 1975, was marked by relatively low mean suspended solids, turbidity, and chemical oxygen demand (COD) values (see figs. 2-4). December was a transition month, as wastewater quality began deteriorating. However, for statistical grouping purposes, December was included in the first segment because effluent quality more closely approximated the quality observed since June. The second time segment, from January through April, was marked by very poor wastewater quality.

Tables 2 and 3 summarize all disinfection data accumulated in each time segment. Geometric mean coliform densities computed over each entire time segment are shown, along with the corresponding standard deviations. The dechlorinated effluent contained no detectable chlorine residual. Ozone residuals were not measured because of the lack of a reliable and quantitative method of analysis.

Mean total and fecal coliform numbers in the nondisinfected effluent were significantly higher ($p \gtrsim 0.99$) in the first interval than in the second. Further, when the mean coliform densities in the unfiltered, nondisinfected

Table 2. REDUCTION OF COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, BROMINE CHLORIDE, AND OZONE JUNE THROUGH DECEMBER, 1975

Treatment	Disinfectant Concentration (mg/l)			Total Coliform Density (MPN/100 ml)				Fecal Coliform Density (MPN/100 ml)			
	Mean Dose	Residual Concentration									
		Mean	S.D.	No. of Samples	Log Mean MPN	S.D.	Geom. Mean MPN	No. of Samples	Log Mean MPN	S.D.	Geom. Mean MPN
Nondisinfected				119	6.48	1.45	3.0×10^6	117	6.08	0.54	1.2×10^6
Chlorinated	4.4	2.0[a]	0.3	124	2.15	0.72	140	122	1.76	0.64	58
Dechlorinated	b			121	2.26	0.61	180	118	1.73	0.59	54
Chlorobrominated	4.2	0.5[c]	0.2	100	2.45	0.76	280	100	1.93	0.82	86
Ozonated	7.4	d		116	2.30	0.68	200	109	1.84	0.62	69

[a] Residual concentration after 30 minutes contact

[b] SO_2 dosage was set to maintain an excess sulfite residual (refer to Section III)

[c] Residual concentration after 5 minutes contact

[d] Residual not measured

Table 3. REDUCTION IN COLIFORM NUMBERS BY CHLORINE, CHLORINE FOLLOWED BY DECHLORINATION, BROMINE CHLORIDE, AND OZONE JANUARY THROUGH APRIL, 1976

Treatment	Disinfectant Concentration (mg/1)			Total Coliform Density (MPN/100 ml)				Fecal Coliform Density (MPN/100 ml)			
	Mean Dose	Residual Concentration Mean	Residual Concentration S.D.	No. of Samples	Log Mean MPN	S.D.	Geom. Mean MPN	No. of Samples	Log Mean MPN	S.D.	Geom. Mean MPN
Nondisinfected				72	6.26	0.40	1.8×10^6	69	5.91	0.45	8.2×10^5
Chlorinated	8.6	2.1	0.7	71	2.32	0.63	210	69	2.00	0.62	100
Dechlorinated	b			62	2.76	0.74	570	63	1.92	0.77	84
Chlorobrominated	8.1	0.6[c]	0.7	47	2.76	0.89	570	44	1.81	1.05	64
Ozonated	8.8	d		69	3.95	0.69	9000	64	3.69	1.01	4900

[a] Residual concentration after 30 minutes contact

[b] SO_2 dosage was set to maintain an excess sulfite residual (refer to Section III)

[c] Residual concentration after 5 minutes contact

[d] Residual not measured

effluent were compared with the levels in the filtered, nondisinfected effluent (not shown in Tables 2 and 3), no significant difference between the means was noted. Thus, pressure filtration of the effluent did not result in a significant removal in bacterial numbers.

The chlorine residual after 30 minutes contact time was maintained at greater than 1.5 mg/l for the duration of the project. The bromine chloride residual after five minutes contact time was planned to be maintained at 0.4 to 0.5 mg/l throughout the project. To maintain these constant residual levels, dosage was paced manually with demand from readings taken on Fisher-Porter continuous chlorine residual analyzers (see Section III) and from grab samples analyzed amperometrically. However, it was found that the fluctuations in the BrCl residual analyzer were too wide to enable continuous maintenance of the desired residual level, presumably due to the instability of BrCl in the presence of reducing agents. Therefore, it was assumed that maintenance of any residual BrCl after five minutes contact time would result in the desired disinfection efficiency.

Halogen dosage was recorded by two different methods. The data appearing in Tables 2 and 3 were derived from cylinder weight loss readings. This method gives the mean halogen dosage for the duration of each time interval. The other method involved taking gas and liquid flow rate readings from rotameters located in the flow pacing equipment. Prior to each sample collection, the halogen dosage was adjusted based on the measured halogen residuals and the dosage recorded. When these data were collected and analyzed, the resulting means were quite different from those depicted in Tables 2 and 3. Thus, the mean chlorine and bromine chloride dosages in the first interval were 6.8 mg/l and 4.5 mg/l, respectively, by the rotameter method (compare with 4.4 mg/l for Cl_2 and 4.2 mg/l for BrCl in Tables 2 and 3). In the second interval, mean Cl_2 and BrCl dosages were 10.1 mg/l and 13.4 mg/l, respectively, measured by the rotameter method (compare with 8.6 mg/l for Cl_2 and 8.1 mg/l for BrCl in Tables 2 and 3).

The precision and accuracy of these measurements were dependent upon several factors. The rotameter floats tended to accumulate debris, causing a change in float density and adherence to the rotameter walls. Gas leaks occasionally developed. Disinfectant dosage control after normal working hours was often less than adequate because of other operator responsibilities. Daily weight-loss readings were recorded from an inherently inaccurate cylinder scale. Thus, the dosage data reported above represent approximate means of fairly imprecise readings and should only be construed as gross estimates of the true dosage rates applied.

It is evident from Tables 2 and 3 that wastewater quality severely deteriorated in the second time interval. Both primary and secondary clarifiers were overloaded with solids, which could not be removed fast enough to prevent a chronic solids carry over. The monthly mean effluent suspended solids and COD levels in this time interval were more than 100 mg/l and 200 mg/l, respectively. Eventually, the clarifiers went septic, causing additional solids spill-over, as rising gas bubbles carried sludge to the surface.

Figures 5, 6, and 7 illustrate the correlation between total coliform numbers and chlorine residual, bromine chloride residual, and ozone dosage, respectively, in the treated effluents. The correlation coefficients (r values) for Cl_2 and BrCl were significant at the $p > 0.99$ level, due to the large number of data points ($n = 170$, 154, and 81 for Cl_2, BrCl, and O_3, respectively). This indicates that total coliform density was inversely proportional to halogen residual. However, the magnitudes of the corresponding coefficients of determination (r^2 values), which is a measure of the strength of a linear relationship, were quite low (0.055 for Cl_2 and 0.098 for BrCl). This means that only 5.5 and 9.8 percent of the variability in coliform densities in the treated effluents were accounted for by the values of the chlorine and bromine chloride correlation coefficients, respectively. Of course, some of the variation in effluent coliform levels was undoubtedly due to variation in influent levels, as the latter were not accounted for in the regression analyses. Nevertheless, the trend exists in the data suggesting that lower bacterial numbers will result from higher halogen residuals in the effluent.

Examination of Figure 7 reveals no correlation between total coliform numbers in the treated effluent and ozone dosage. This is not unexpected, since ozone dosage could not be controlled by residual. In fact, much of the data recorded in the second time segment (i.e., when wastewater quality was poor) were obtained under conditions when the ozone generator, which was operating at maximum capacity, could not produce sufficient ozone to meet the demand of the wastewater.

Figures 8 to 16 illustrate the effects of turbidity, chemical oxygen demand, and suspended solids on disinfectant dosage necessary to maintain a given residual (except in the case of ozone, where no residual could be maintained). It is clear that, in all cases involving the halogens, as the levels of key water quality parameters increased, the halogen dosage had to be raised in order to maintain the desired residual concentrations necessary to meet project disinfection standards. The correlation coefficients were all statistically significant at the $p > 0.99$ level. However, no such conclusion can be reached with ozone, because the correlation coefficients and the corresponding coefficients of determination were too low and the Y-intercept values were too high to provide a meaningful interpretation of the data. This again is not unexpected because of the lack of adequate control of ozone dosage as a function of demand.

A two-way analysis of variance was performed on all coliform data to test two null hypotheses: (1) there is no significant difference among treatment means (i.e., chlorinated, dechlorinated, ozonated, and chlorobrominated effluents) with respect to geometric mean coliform densities; (2) there is no significant difference in geometric mean coliform densities in the treated effluents between the two time segments (i.e., June-December, and January-April). Results from this analysis revealed that the mean coliform densities among treatments (and, thus, disinfection efficiencies) were significantly different ($p > 0.99$). Furthermore, mean coliform densities in all effluents in the first time segment were significantly different ($p > 0.99$) from the mean coliform densities in the second time segment. This indicates that wastewater quality considerably affected the performance of all disinfectants.

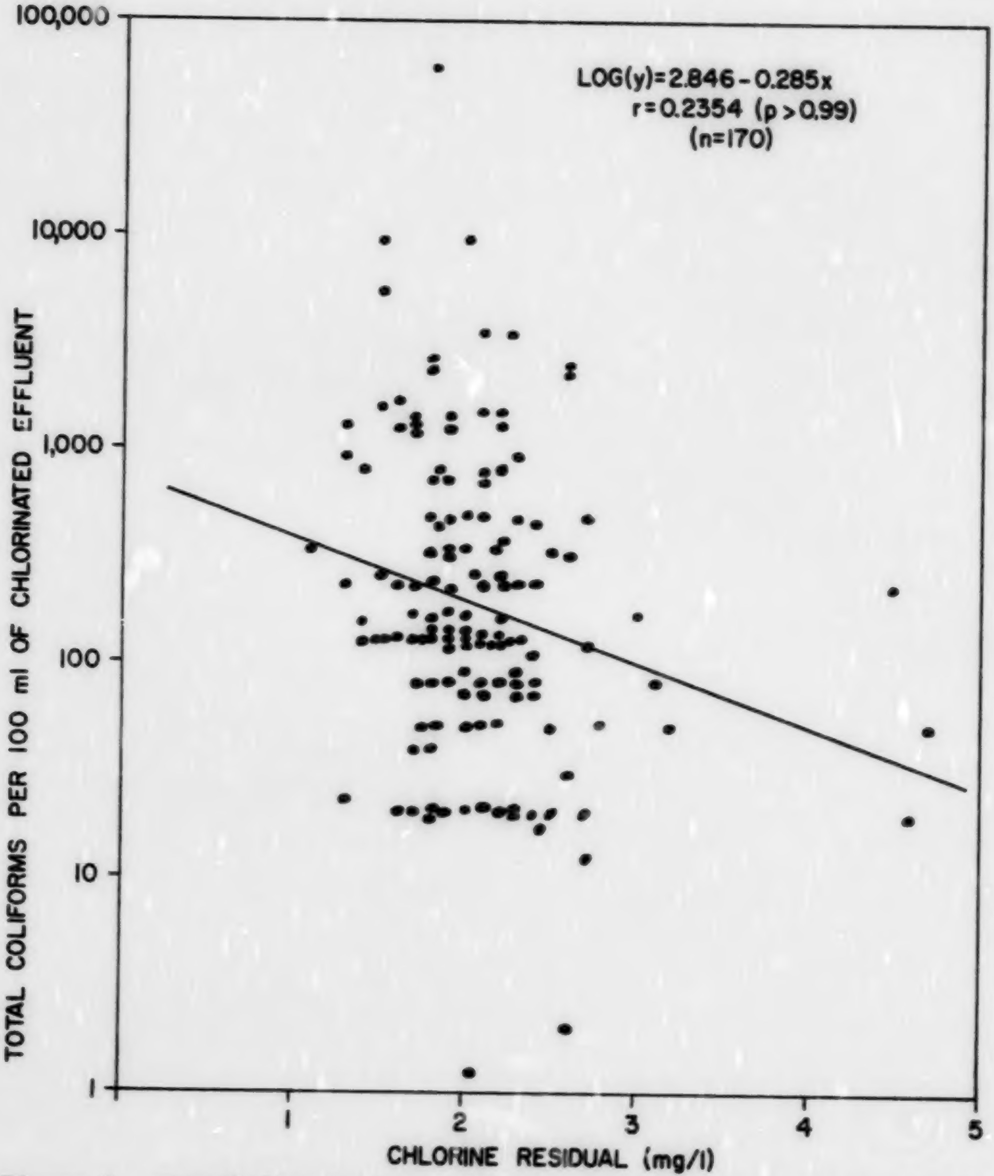


Figure 5. Correlation between chlorine residual concentration (mg/1) and resulting total coliform density (number/100 ml of chlorinated effluent).

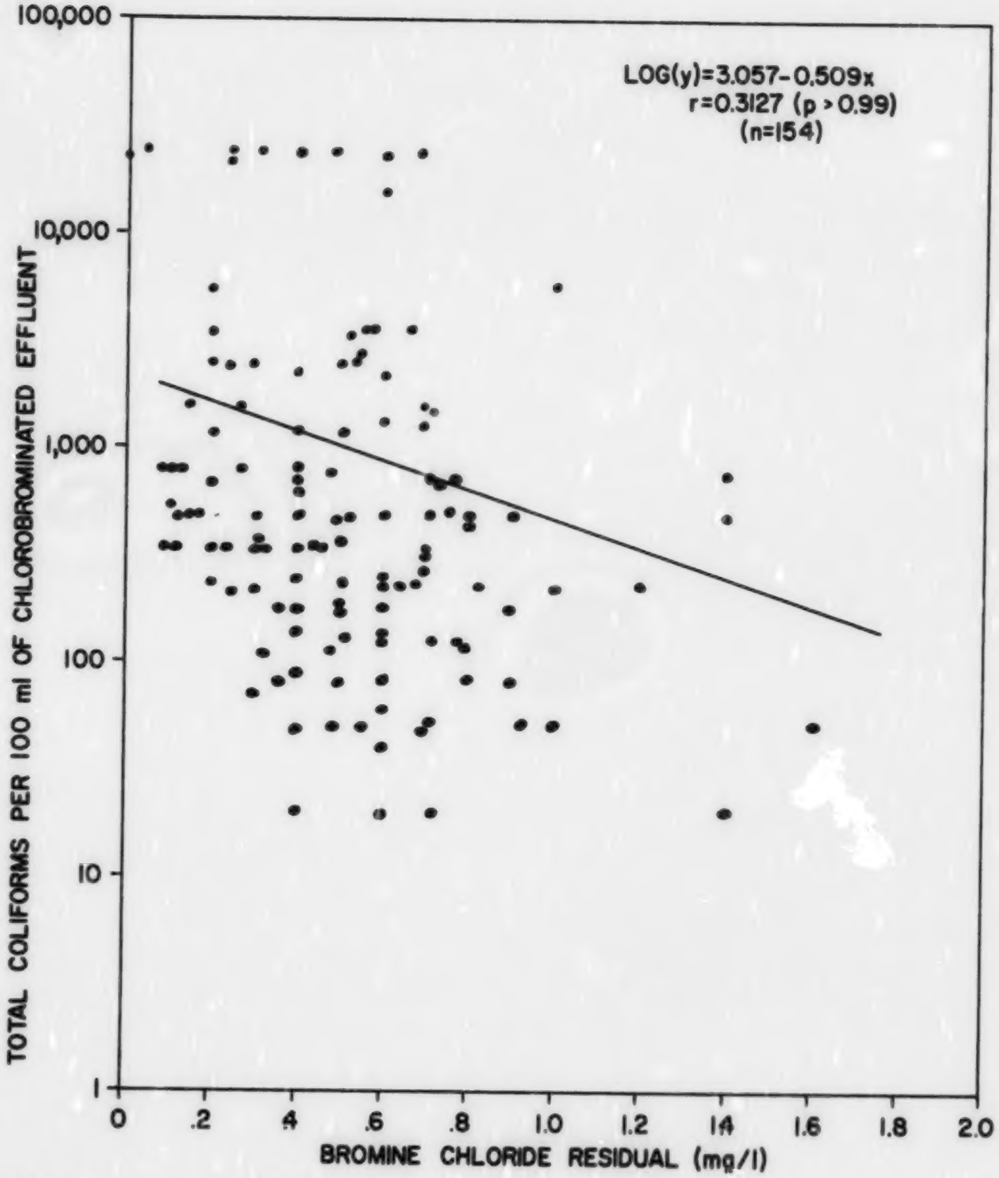


Figure 6. Correlation between bromine chloride residual concentration (mg/l) and resulting total coliform density (number/100 ml of chlorobrominated effluent).

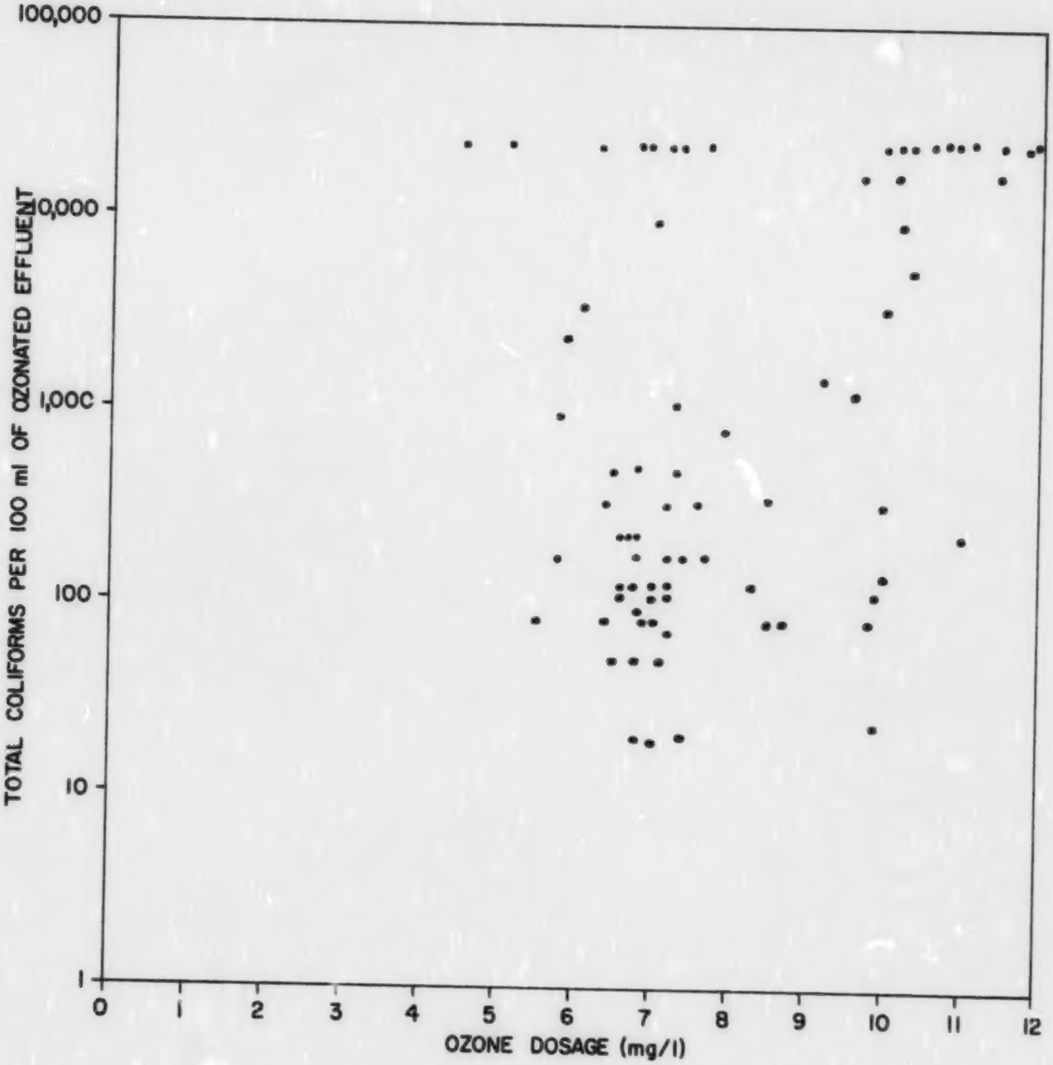


Figure 7. Correlation between ozone dosage (mg/l) and resulting total coliform density (number/100 ml of ozonated effluent).

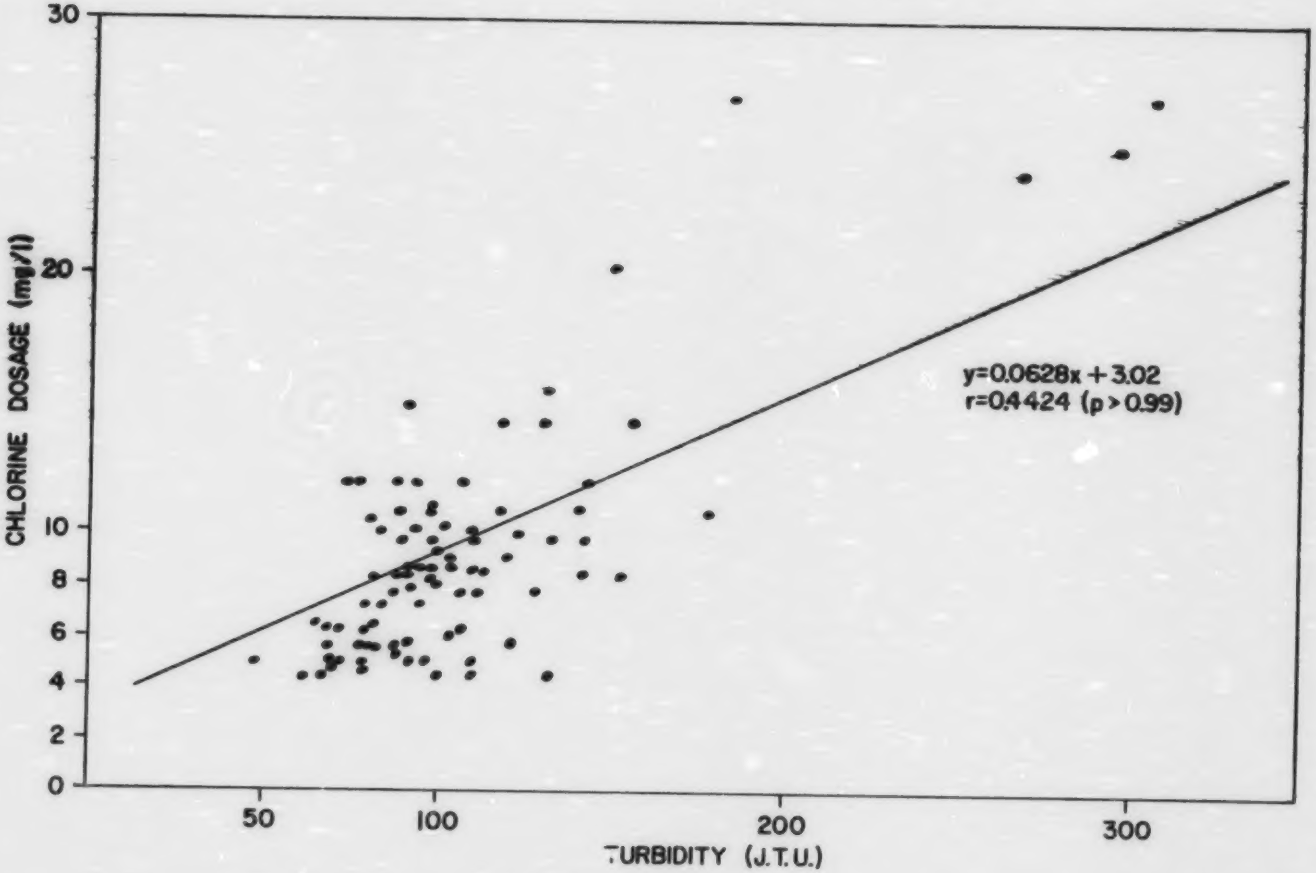


Figure 8. Correlation between turbidity (J.T.U.) and chlorine dosage (mg/l) necessary to maintain a total residual chlorine level > 1.5 mg/l after 30 minutes.

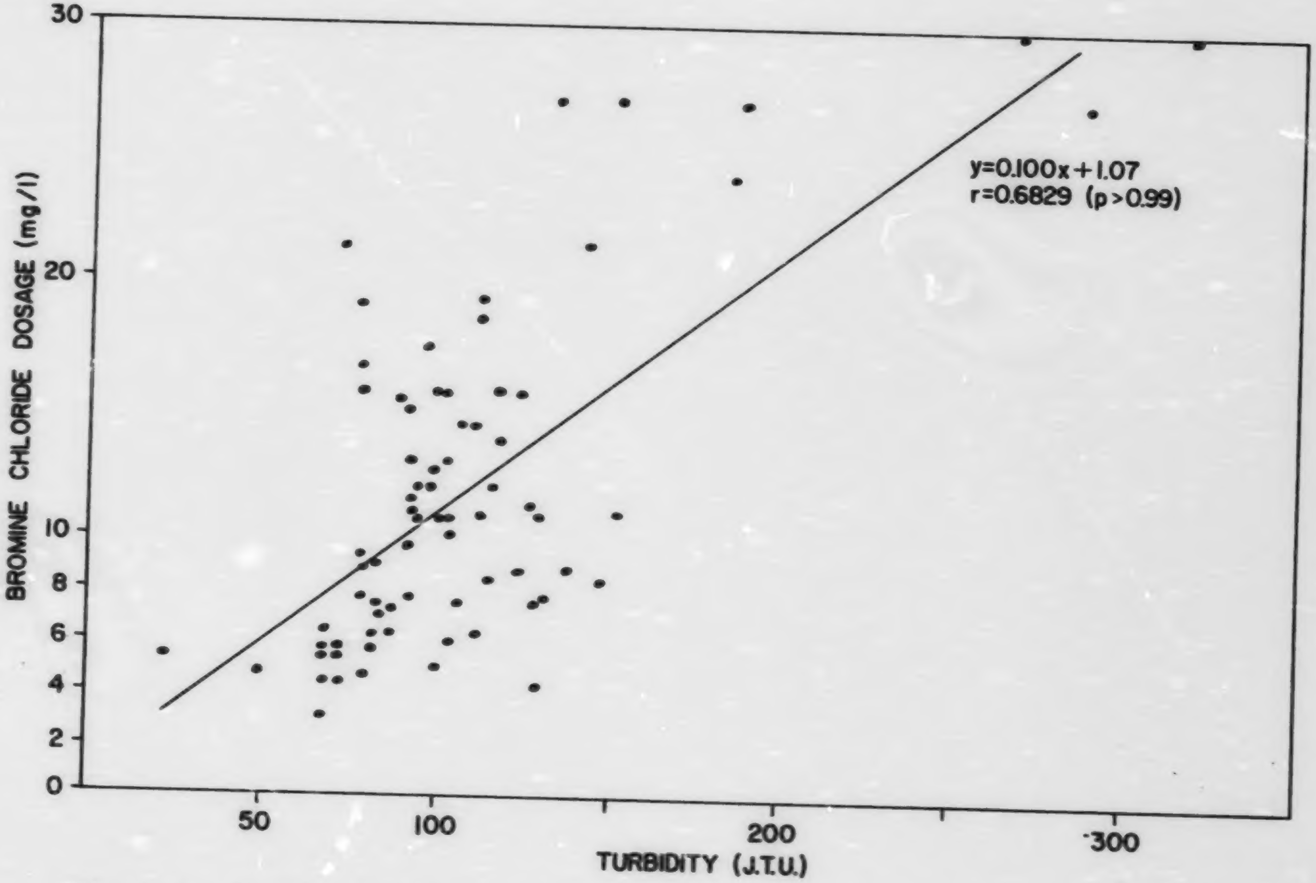


Figure 9. Correlation between turbidity (J.T.U.) and bromine chloride dosage (mg/1) necessary to maintain any titrable residual after 5 minutes.

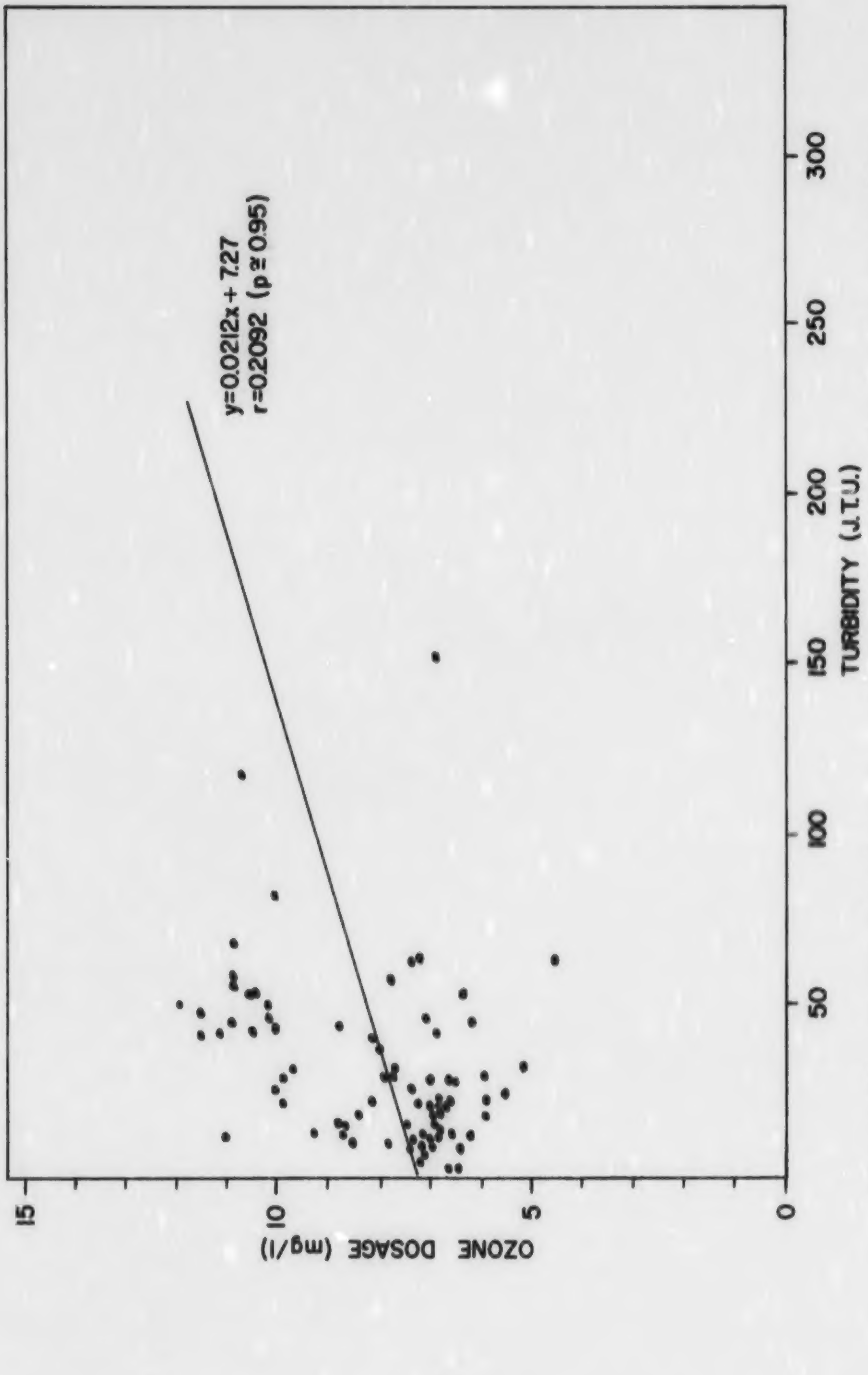


Figure 10. Correlation between turbidity (J.T.U.) and ozone dosage (mg/l).

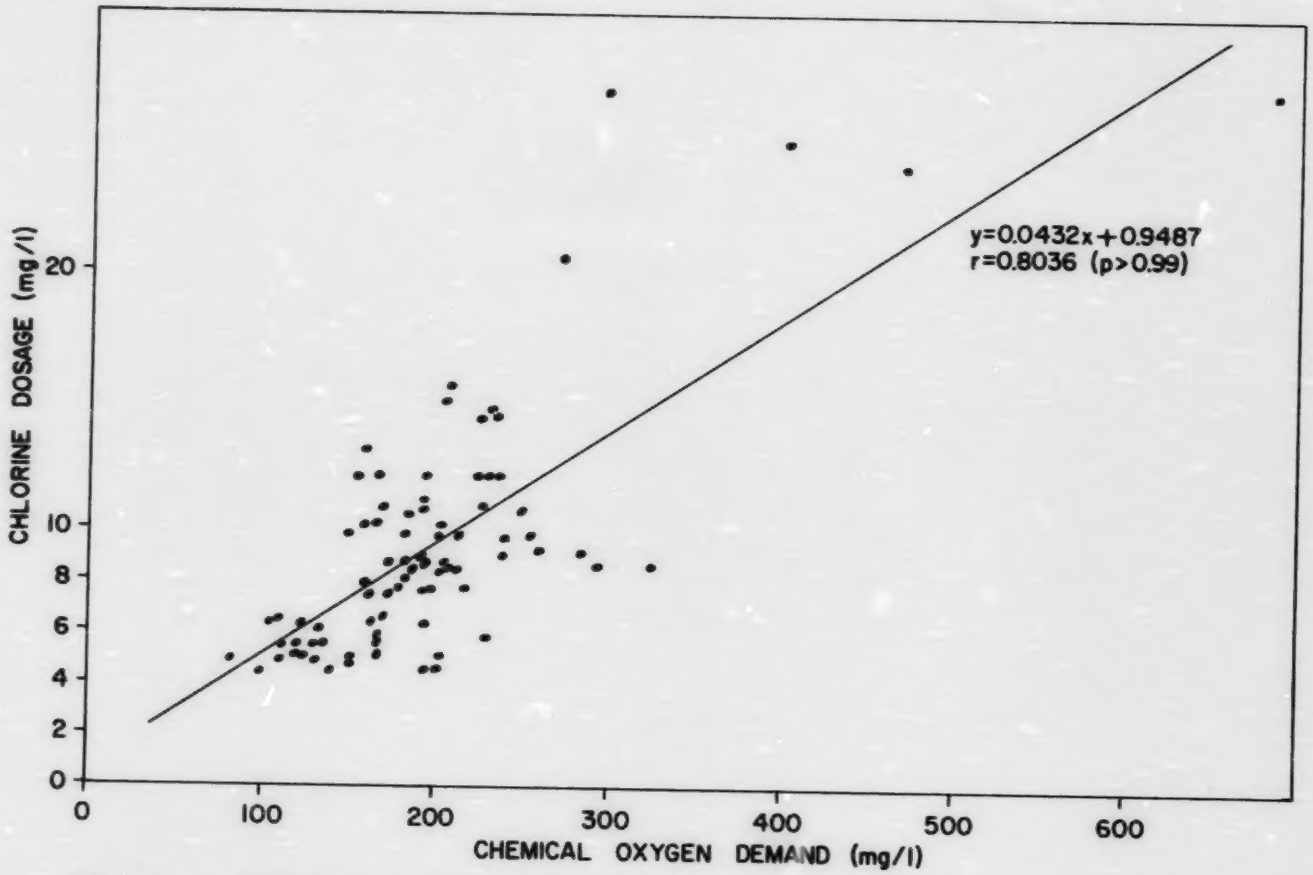


Figure 11. Correlation between chemical oxygen demand (mg/l) and chlorine dosage (mg/l) necessary to maintain a total residual chlorine level >1.5 mg/l after 30 minutes.

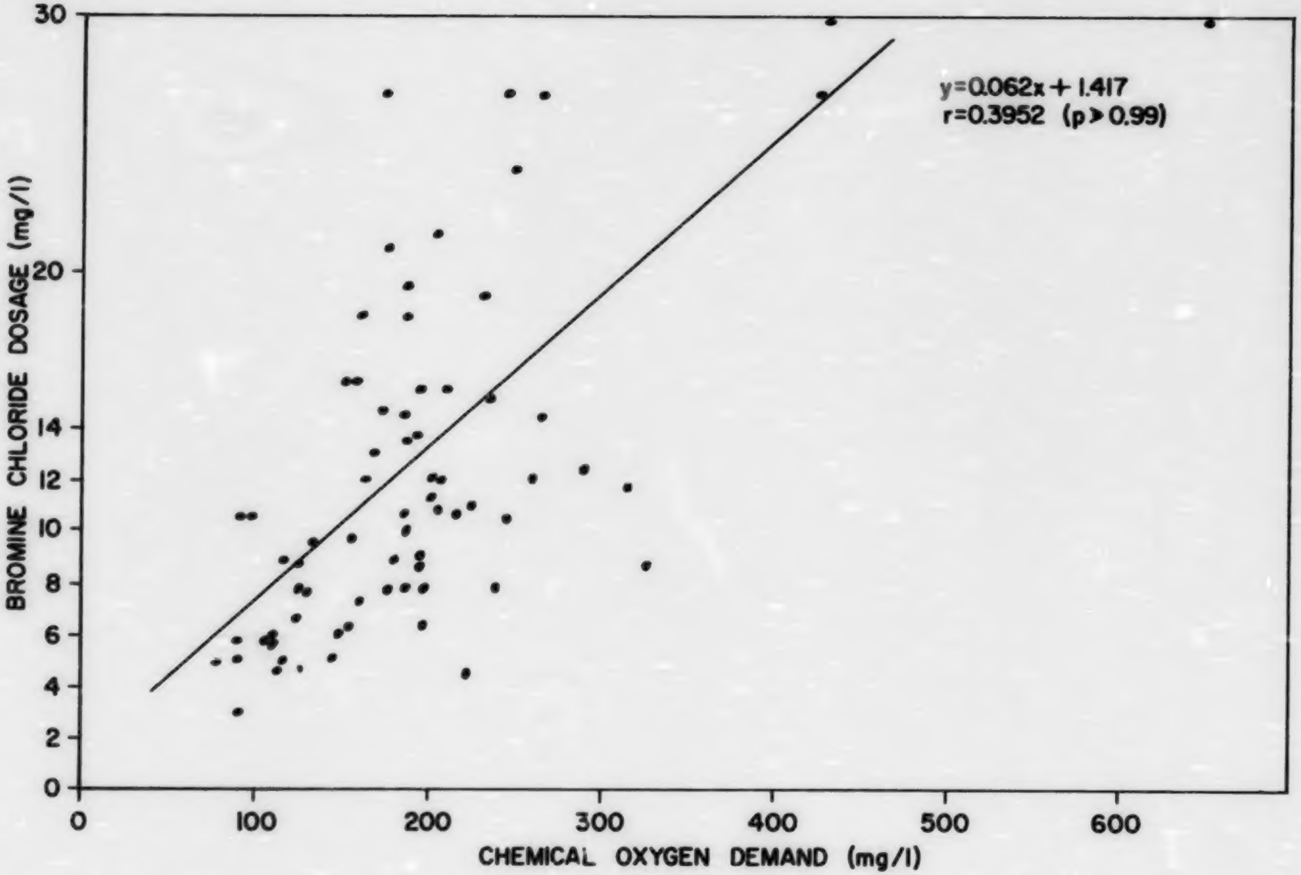


Figure 12. Correlation between chemical oxygen demand (mg/l) and bromine chloride dosage (mg/l) necessary to maintain any titrable residual after 5 minutes.

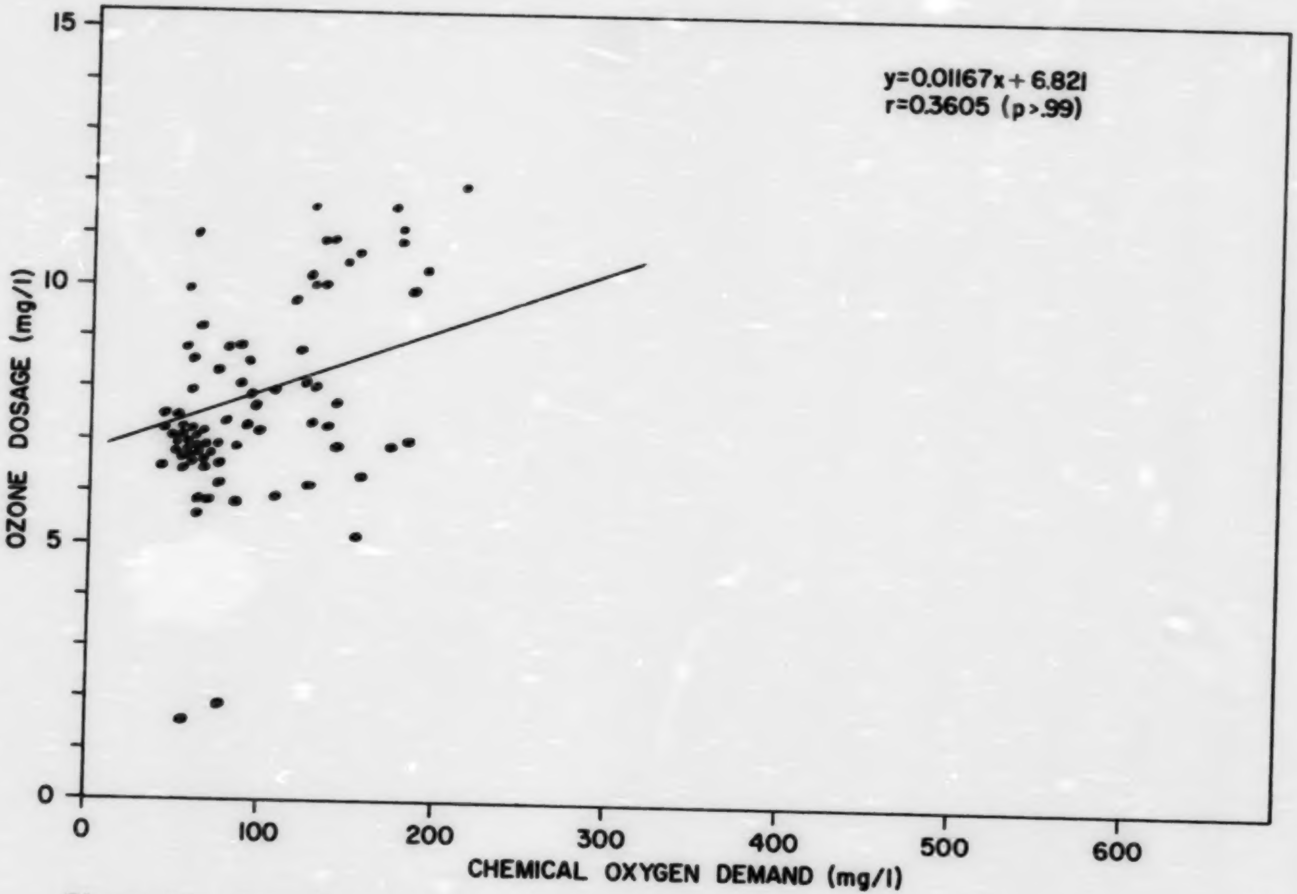


Figure 13. Correlation between chemical oxygen demand (mg/1) and ozone dosage (mg/1).

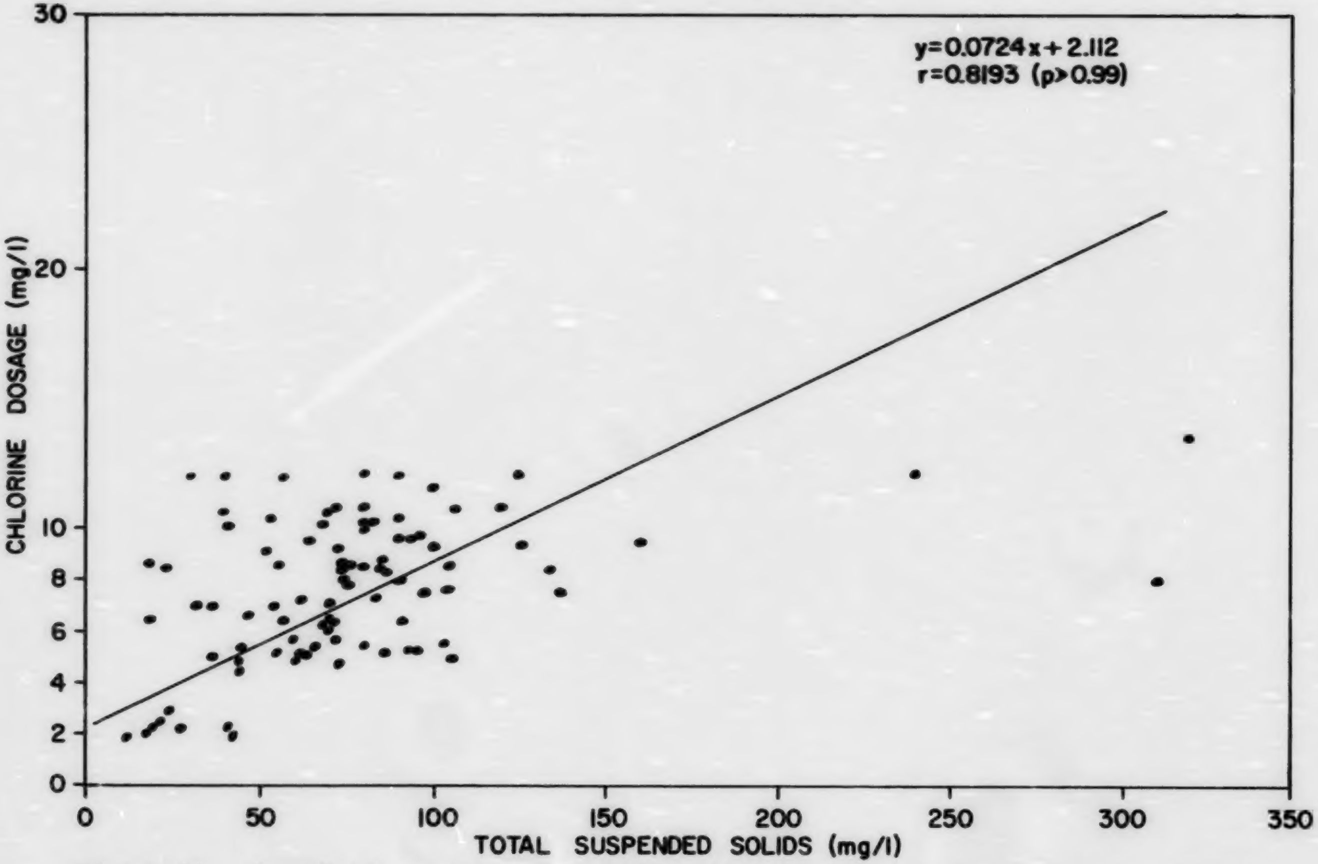


Figure 14. Correlation between suspended solids (mg/1) and chlorine dosage (mg/1) necessary to maintain a total residual chlorine level >1.5 mg/1 after 30 minutes.

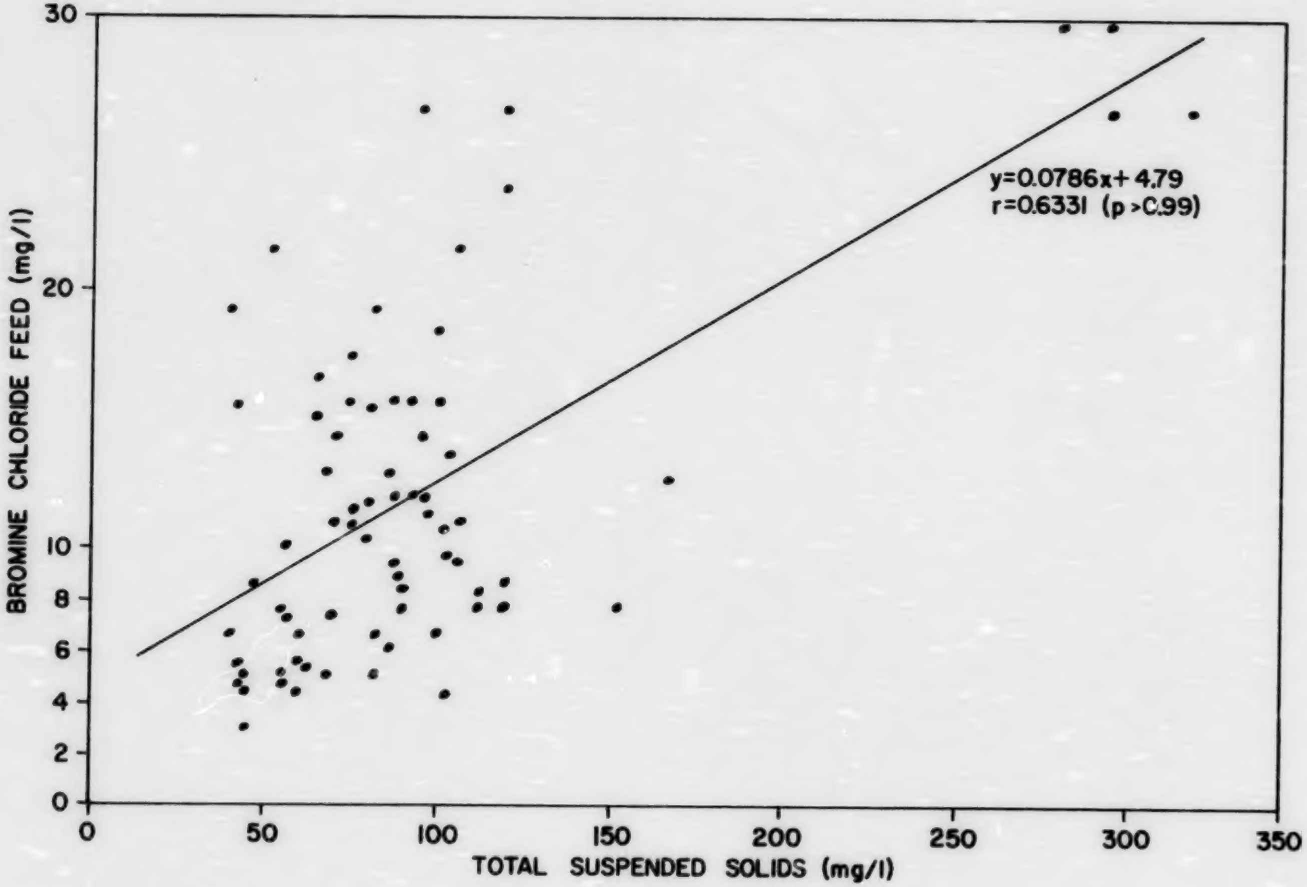


Figure 15. Correlation between suspended solids (mg/1) and bromine chloride dosage (mg/1) necessary to maintain any titrable residual after 5 minutes.

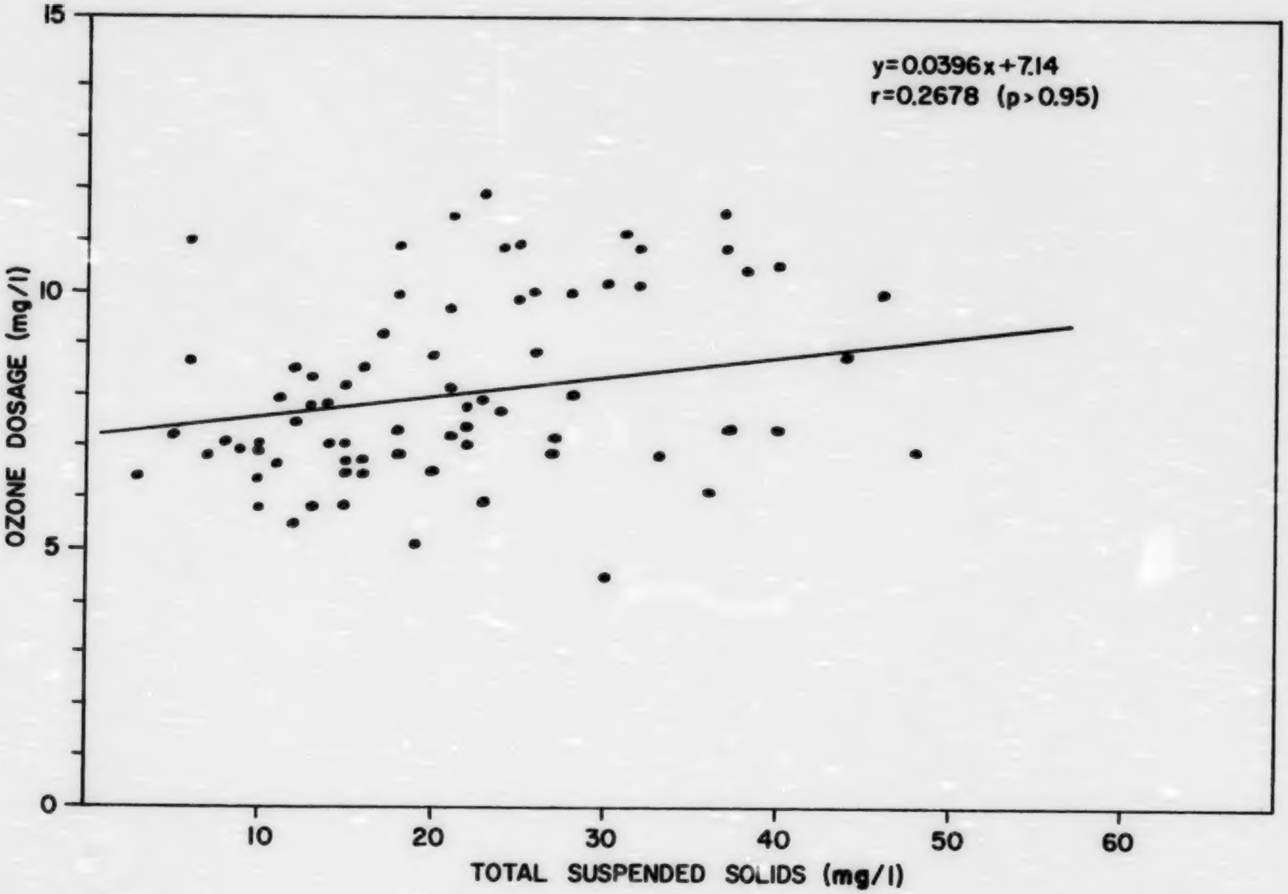


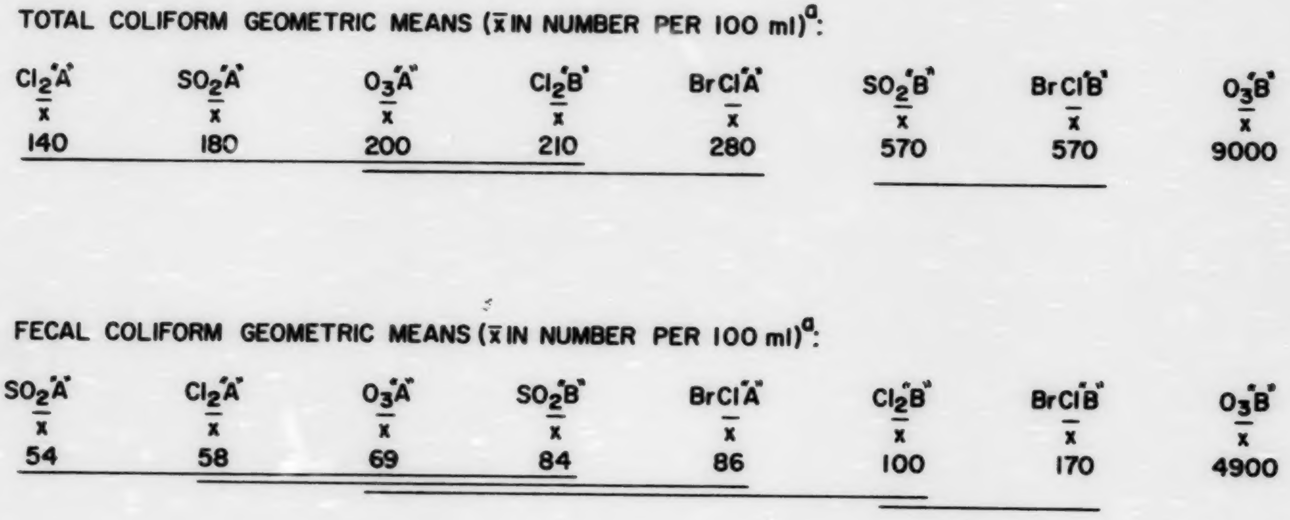
Figure 16. Correlation between suspended solids (mg/1) and ozone dosage (mg/1).

However, the analysis of variance also indicated a significant interaction between treatments and time segments ($p > 0.99$). This means that the differences observed among disinfectants were not consistent in both time segments, suggesting wastewater quality affects performance of each disinfectant in different ways. Indeed, this is not unexpected, since the reactivities of each disinfectant with various demand materials are known to be quite diverse.

Since the analysis of variance only indicated significant differences among treatment means, it did not define which means were significantly different or how many differences there were. To test hypotheses about these differences, a Least Significant Difference test[2] was performed on the same data. Figure 17 illustrates diagrammatically the significant differences in total and fecal coliform geometric means as determined by this method. Any pair of means within the range of any one line below it is not significantly different ($p > 0.95$). In general, results from the fecal coliform data were similar to those from the total coliform data. Considering both total and fecal coliform means, each mean in the first interval ("A") was lower than its counterpart in the second interval ("B"). This difference was statistically significant ($p > 0.95$) for all total coliform treatments except chlorination and all fecal coliform treatments except dechlorination.

In general, fewer differences among treatment means occurred in the first time segment than in the second. Thus, in the first interval, only two pairs of total coliform means were significantly different (i.e., Cl_2/BrCl and SO_2/BrCl, the BrCl being greater than both), and only one pair of fecal coliform means displayed a difference (i.e., SO_2/BrCl, the BrCl again being the greater mean). However, in the second interval, most coliform means were significantly different. Thus, only one pair of total coliform means was not significantly different (i.e., SO_2/BrCl), and only two pairs of fecal coliform means exhibited no significant difference (i.e., SO_2/Cl_2 and Cl_2/BrCl). Both the total and fecal coliform means from the ozone treatment in the second interval were higher ($p > 0.99$) than all other treatment means in both intervals. It appears that chlorine is least affected by changes in wastewater quality, followed by bromine chloride and then ozone. This is undoubtedly due to the inherently greater ability to control chlorine dosage as a function of chlorine demand and suggests the need to develop better means of controlling bromine chloride and ozone in a similar fashion to chlorine.

Regarding the untreated (i.e., nondisinfected) effluent, a t-test was performed to determine if there was a significant difference in total and fecal coliform levels between the two time segments. Results indicated that both total and fecal coliform means were significantly greater ($p > 0.99$) in the first interval than in the second. Thus, the gross deterioration in organic quality of the Wyoming effluent from January to April, 1976, compared to June to December, 1975, was not paralleled by a corresponding deterioration in bacteriological quality.


TOTAL COLIFORM GEOMETRIC MEANS ($\bar{x}$ IN NUMBER PER 100 ml)[a]:
Cl_2"A" $\bar{x}$ 140
SO_2"A" $\bar{x}$ 180
O_3"A" $\bar{x}$ 200
Cl_2"B" $\bar{x}$ 210
BrCl"A" $\bar{x}$ 280
SO_2"B" $\bar{x}$ 570
BrCl"B" $\bar{x}$ 570
O_3"B" $\bar{x}$ 9000
FECAL COLIFORM GEOMETRIC MEANS ($\bar{x}$ IN NUMBER PER 100 ml)[a]:
SO_2"A" $\bar{x}$ 54
Cl_2"A" $\bar{x}$ 58
O_3"A" $\bar{x}$ 69
SO_2"B" $\bar{x}$ 84
BrCl"A" $\bar{x}$ 86
Cl_2"B" $\bar{x}$ 100
BrCl"B" $\bar{x}$ 170
O_3"B" $\bar{x}$ 4900

[a] ANY PAIR OF MEANS ENCLOSED BY THE RANGE OF ANY ONE LINE IS NOT SIGNIFICANTLY DIFFERENT ($p \geq 0.95$)

Figure 17. Diagramatic representation of significant differences (least significant difference, ($p \geq 95\%$)) between total and fecal coliform means from chlorinated (Cl_2), dechlorinated (SO_2), chlorobrominated (BrCl), and ozonated (O_3) effluents taken from June through December, 1975 ("A") and January through April, 1976 ("B").

Frequency of Project Standards Achievement

Table 4 illustrates the frequency that each disinfectant reduced total and fecal coliform densities to project standards within each time interval and then combined for the entire project. In the first interval, all treatments were effective in enabling achievement of both bacteriological standards. In the second interval, all process efficiencies diminished, the most dramatic decrease being exhibited by ozone. The reason for this, as already stated, was that the ozonation system in use did not have the capability to respond to such an extreme increase in demand, as it was already operating at maximum capacity.

It is clear from observation of Table 4 that the number of samples analyzed from each treatment varied. The reason for this was mechanical breakdowns in the test equipment. The BrCl dosing unit and accompanying residual analyzer frequently malfunctioned. Thus, when such an occurrence took place, either a sample was not collected at all, or the analysis of a sample obtained under such conditions was subsequently excluded from the overall evaluation. If the number of samples collected were not shown in Table 4, one would receive the impression that BrCl was equally effective as Cl_2 in attainment of desired bacteriological goals, when in reality the only data included in the evaluation were those obtained when the BrCl system was functioning properly (approximately 80 percent of the time in the first interval, and 66 percent of the time in the second interval). The problem with the BrCl dosing system was the accumulation of solid and semi-solid contaminants in the evaporator, resulting in blockage of flow of the BrCl gas. This system malfunctioned substantially more often than any other system, even after a newly-designed dosing apparatus had been introduced in February, 1976.

The ozone generation system operated better than it had in the previous study at Grandville. Two minor electrical malfunctions occurred in October, 1975, and in February, 1976. However, little experimental time was lost due to the presence of a back-up ozone generation system. Two serious malfunctions in the air compressor occurred in September, 1975, and in March, 1976, resulting in a moderate amount of down time.

Observations of Data on a Monthly Basis

To inspect the temporal relationships of the performance of the various disinfection processes in a more typical fashion, mean coliform data are presented in Figures 18 to 21 in terms of monthly intervals. Additional information is presented in Appendices 1-5, which show sample sizes, standard deviations, and arithmetic and geometric means.

Figures 18 and 19 show the monthly geometric means of total and fecal coliform densities, respectively, in all test streams. All streams exhibit moderately stable monthly total and fecal coliform means for the first seven months of the study (through December, 1975).

Starting in January, 1976, and continuing until the end of the experimental period, a deterioration in bacteriological quality in all effluent streams

Table 4. FREQUENCY[a] THAT DAILY SAMPLES OF DISINFECTED EFFLUENT[b] ACHIEVED PROJECT BACTERIOLOGICAL STANDARDS

Time Interval	Total Coliforms % of Samples Below 1000 per 100 ml				Fecal Coliforms % of Samples Below 200 per 100 ml			
	Cl_2	SO_2	BrCl	O_3	Cl_2	SO_2	BrCl	O_3
June to December, 1975	88 (124)[c]	89 (121)	85 (100)	86 (116)	81 (122)	84 (118)	77 (100)	84 (109)
January to April, 1976	83 (71)	69 (62)	72 (47)	12 (69)	68 (69)	59 (63)	68 (44)	16 (64)
Combined	86 (195)	83 (183)	81 (147)	58 (185)	77 (191)	75 (181)	74 (144)	59 (173)

[a] Frequency calculated as number of samples which met coliform standards divided by total number of samples times 100%

[b] For disinfectant concentrations, refer to Tables 2 and 3

[c] Number of samples in parentheses

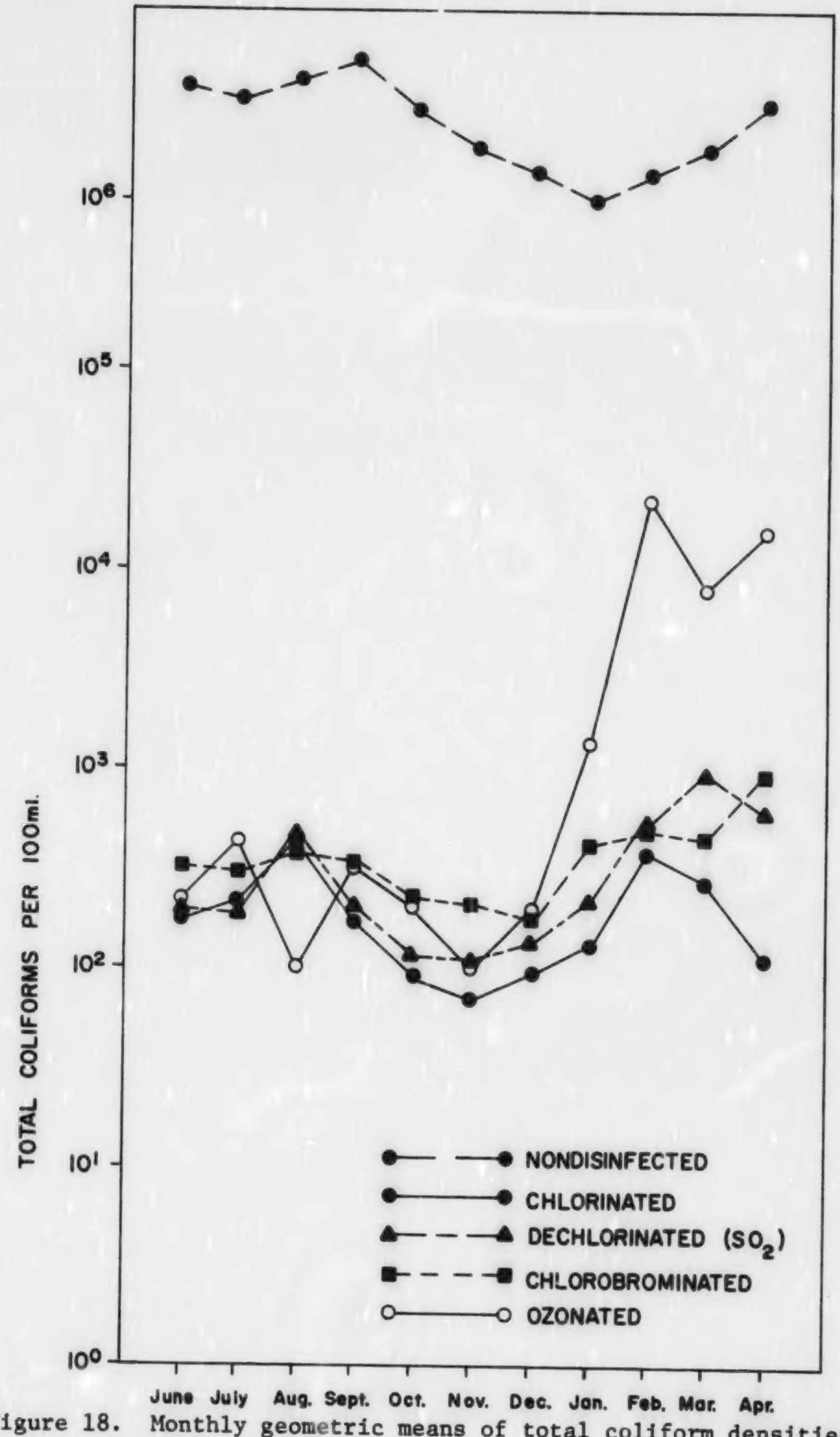


Figure 18. Monthly geometric means of total coliform densities (MPN) for each stream.

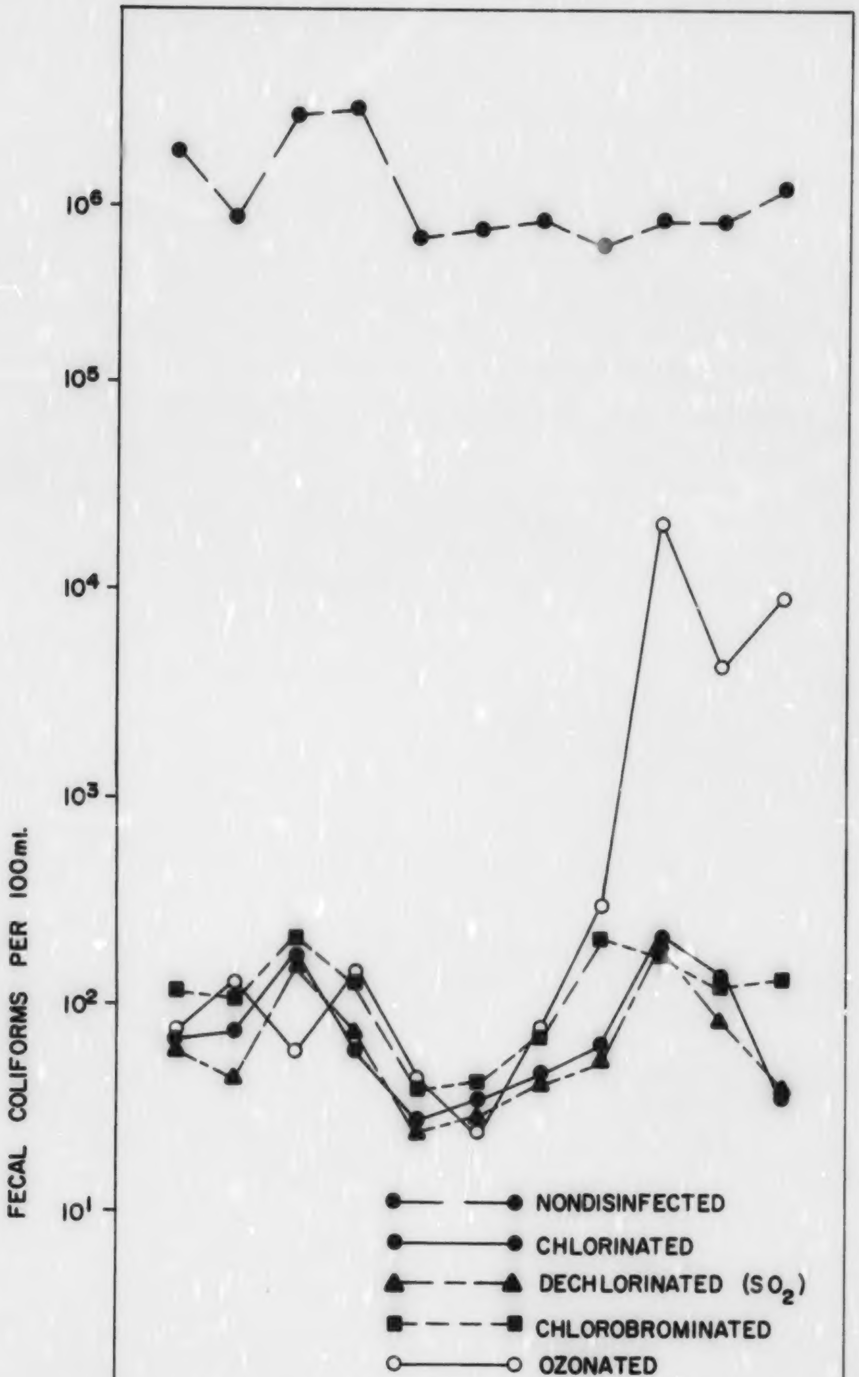


Figure 19. Monthly geometric means of fecal coliform densities (MPN) for each stream.

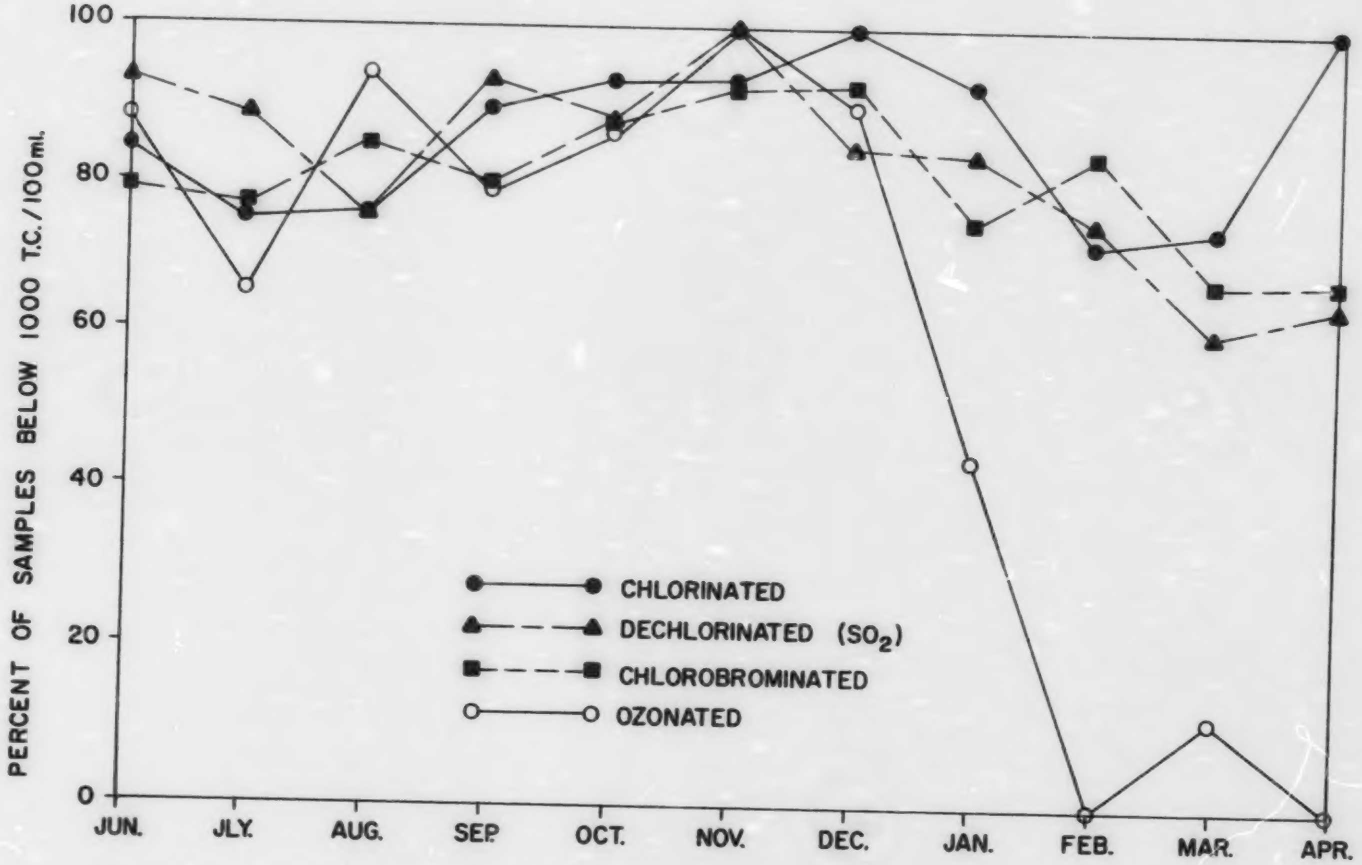


Figure 20. Monthly frequencies that daily samples of disinfected effluent achieved the project total coliform standard of ≤1000 organisms per 100 ml sample.

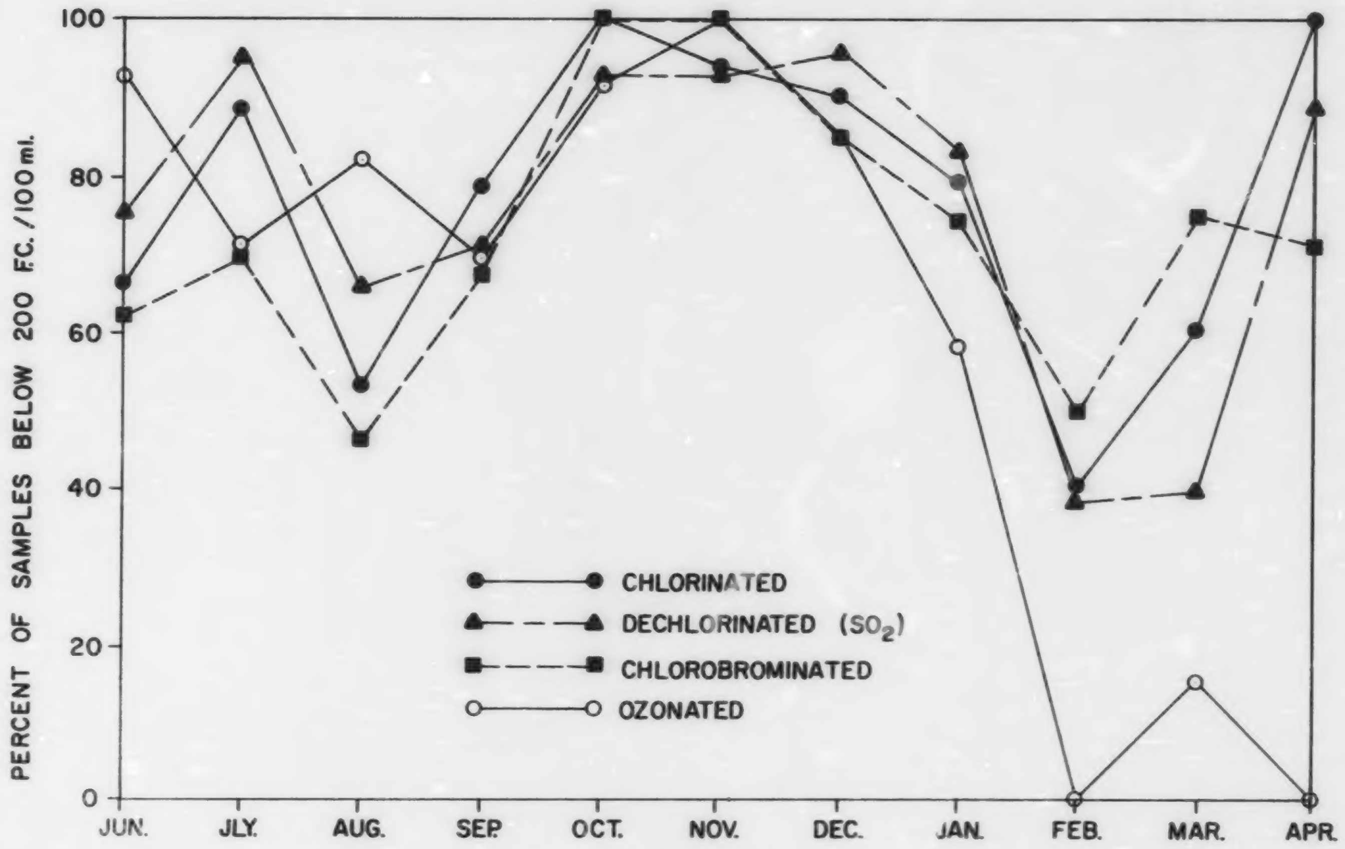


Figure 21. Monthly frequencies that daily samples of disinfected effluent achieved the project fecal coliform standard of ≤200 organisms per 100 ml sample.

except the nondisinfected is quite evident. The increase in monthly coliform means is particularly marked in the ozonated effluent. Coliform levels in the chlorobrominated stream were generally higher than the chlorinated and dechlorinated streams, but not in every instance. Total coliform densities in the dechlorinated stream were consistently higher than those in the chlorinated stream, particularly from January, 1976, to April, 1976, whereas the converse is true in the fecal coliform population. This fact is further supported by the previously discussed Least Significant Difference Test (Figure 17), in which it was determined that the mean total coliform density in the dechlorinated stream (SO_2 "B") was significantly higher ($p > 0.95$) than the chlorinated stream (Cl_2 "B"), while the mean fecal coliform level in the dechlorinated stream (SO_2 "B") was significantly lower than the chlorinated stream (Cl_2 "B").

Figures 20 and 21 present the frequency, on a monthly geometric mean basis, that samples from each treatment met project disinfection standards. These data closely parallel those discussed above.

Summary

Duration of the project was eleven months. During the first seven months of the project, wastewater quality was satisfactory. As winter temperatures approached, upsets occurred in the biological treatment system, giving rise to increased organic demand in the effluent prior to disinfection. Disinfection efficiencies of all experimental processes were affected to varying degrees by the deteriorating conditions. Bromine chloride and ozone displayed the greatest sensitivities to these shifts in demand. Excellent correlations were found between chlorine and bromine chloride dosages necessary to maintain given residuals and turbidity, suspended solids, and chemical oxygen demand. No correlation was found between ozone dosage and organic demand due not only to the lack of an adequate means of controlling dosage in accordance with such fluctuations, but also to the incapability of the ozone generation system to meet the higher dosage requirements.

Malfunctions in the BrCl and O_3 dosing systems occurred with greater frequency than in the chlorination and dechlorination systems, suggesting the need to improve the engineering capability of these newer processes, as well as to develop better means of controlling dosage as a function of demand. Thus, application of these newer technologies to wastewater disinfection is supportable in that they have demonstrated the capability to reduce total and fecal coliform densities to acceptable levels. Nevertheless, they have also demonstrated a tendency to require more maintenance and repairs due to incomplete technology development.

Appendix 1

COLIFORM DENSITIES OF NONDISINFECTED EFFLUENT
JUNE, 1975 THROUGH APRIL, 1976
(number/100 ml)

	Total Coliform Density		Fecal Coliform Density	
	No. of Samples	Geometric Mean	No. of Samples	Geometric Mean
June	18	3.9×10^6	16	1.7×10^6
July	14	3.3×10^6	14	9.6×10^5
August	17	4.0×10^6	17	2.9×10^6
September	17	5.9×10^6	17	2.9×10^6
October	18	2.8×10^6	18	5.3×10^5
November	16	1.8×10^6	16	6.2×10^5
December	19	1.5×10^6	19	8.2×10^5
January	14	1.0×10^6	14	4.8×10^5
February	19	1.6×10^6	17	8.2×10^5
March	23	1.8×10^6	23	8.4×10^5
April	16	3.0×10^6	15	1.3×10^6

Appendix 2

COLIFORM DENSITIES OF CHLORINATED EFFLUENT
JUNE, 1975 THROUGH APRIL, 1976
(number/100 ml)

	Total Coliform Density		Fecal Coliform Density	
	No. of Samples	Geometric Mean	No. of Samples	Geometric Mean
June	18	160	17	68
July	16	210	16	73
August	17	390	17	180
September	18	170	18	58
October	18	91	18	29
November	16	70	16	35
December	20	95	20	46
January	14	140	14	66
February	18	380	17	220
March	23	270	23	160
April	16	110	15	34

Appendix 3

COLIFORM DENSITIES OF DECHLORINATED EFFLUENT
JUNE, 1975 THROUGH APRIL, 1976
(number/100 ml)

	Total Coliform Density		Fecal Coliform Density	
	No. of Samples	Geometric Mean	No. of Samples	Geometric Mean
June	18	180	16	59
July	16	180	16	44
August	17	450	17	180
September	18	200	17	76
October	18	120	18	26
November	15	120	15	31
December	19	140	19	47
January	12	230	12	55
February	16	550	16	220
March	20	1000	20	88
April	14	590	15	39

Appendix 4

COLIFORM DENSITIES OF CHLOROBROMINATED EFFLUENT
JUNE, 1975 THROUGH APRIL, 1976
(number/100 ml)

	Total Coliform Density		Fecal Coliform Density	
	No. of Samples	Geometric Mean	No. of Samples	Geometric Mean
June	14	320	13	120
July	13	310	13	110
August	13	350	13	220
September	15	370	15	140
October	17	250	18	42
November	15	220	15	41
December	13	190	13	70
January	8	420	8	210
February	12	500	10	180
March	12	450	12	140
April	15	920	14	160

Appendix 5

COLIFORM DENSITIES OF OZONATED EFFLUENT
JUNE, 1975 THROUGH APRIL, 1976
(number/100 ml)

	Total Coliform Density		Fecal Coliform Density	
	No. of Samples	Geometric Mean	No. of Samples	Geometric Mean
June	17	210	14	67
July	17	430	17	130
August	17	98	17	60
September	14	320	13	130
October	15	210	12	47
November	16	110	16	27
December	20	200	20	79
January	14	1600	12	300
February	20	23000	18	21000
March	19	7900	19	4300
April	16	16000	15	9700

REFERENCES

1. Standard Methods for the Examination of Water and Wastewater. 13th ed. New York, American Public Health Association, 1971. 874 pp.

2. Sokal, R. R., and F. J. Rohlf. Biometry. San Francisco, W. H. Freeman and Co., 1969. 368 pp.

SECTION V

LIFE CYCLE RESIDUAL TOXICITY STUDIES

INTRODUCTION

The residual toxicity of chlorinated wastewater effluent has been demonstrated in several recent studies.[1-8] As a result of these findings, attention has been given to either neutralizing chlorine residuals in treated effluents or replacing chlorine with a disinfectant which has no residual toxicity. The former approach has shown that residual chlorine toxicity can be eliminated through the use of sulfur dioxide, [1,8] bisulfite,[3] or sodium thiosulfate.[6] Among the alternative processes being considered for wastewater disinfection are ozonation [8-10] and chlorobromination.[8,11,12]

The life cycle study reported herein was designed to determine whether various chemical disinfectants, applied to a trickling filter effluent derived from wastewater containing a large proportion of industrial wastes, imparted a long-term residual toxic effect to fish. Thus, the toxicity of parallel effluent streams treated with chlorine (Cl_2), sulfur dioxide (SO_2) dechlorination, bromine chloride (BrCl), or ozone (O_3) and a nondisinfected control stream was examined. The fathead minnow, Pimephales promelas, was the subject of these tests.

METHODS AND MATERIALS

Most of the bioassay methods and materials were similar to those described in the Grandville report.[8] The following paragraphs include only highlights and aspects unique to the Wyoming study site.

Chemical Analyses

Residual Cl_2, BrCl, O_3, and sulfite (residual SO_2 was measured as sulfite) were measured bi-weekly in the respective effluent storage tanks and daily in one aquarium containing the highest effluent concentration (see Vol. I for methods). Heavy metal analyses were performed weekly on effluent samples which were stored from the preceeding seven days. A Perkin-Elmer model 403 Atomic Absorption Spectrophotometer was used for these determinations. Also, on Mondays through Fridays, three 8-hour composite samples from the preceeding day were tested for cyanide concentrations using the colorimetric pyridine-barbituric acid method.[13]

Water Supplies

Detailed descriptions of the treatment site and the effluent flow schemes are presented in Section III of this report. Dilution water was obtained from a well at the study site and had the characteristics shown in Table 5. The pH and conductivity of the well water were 7.4 and 1663 µmhos/cm, respectively.

Table 5. CHARACTERISTICS OF THE DILUTION WATER

Analysis	Concentration in mg/l
Hardness (as $CaCO_3$)	782
Calcium	272
Magnesium	14
Sulfate	545
Chloride	86
Iron (prior to iron filtration)	1.39
NH_3-N	0.60
Alkalinity (as $CaCO_3$)	197
Acidity (as $CaCO_3$)	25

Bioassay Methods

The effluents and dilution (well) water were delivered to headtanks where they were heated to 25C for life cycle bioassays with P. promelas. The residence time in the headboxes was approximately 45 minutes.

With the exception of the chlorinated effluent diluter system, all diluters were calibrated to deliver 100 percent effluent, 100 percent dilution water, and six intermediate nominal effluent concentrations of 50.00, 25.00, 12.50, 6.25, 3.12, and 1.56 percent. The chlorinated effluent diluter was designed to deliver nominal effluent dilutions of 50.00, 25.00, 18.75, 12.50, 6.25, 3.12, and 1.56 percent, and 100 percent dilution water. Lower concentrations of chlorinated effluent were used because of the previously demonstrated toxicity of chlorinated effluent to aquatic life.[1]

The calibration of all life cycle diluters and flow-splitting cells was checked volumetrically each week and adjusted, if necessary, to maintain the proper effluent concentration and turnover time in each test chamber. Due to the generally poor quality effluent, frequent adjustments of all diluters and flow-splitting cells were necessary to maintain proper calibration throughout the entire study. Periodically, it became necessary to completely disassemble and clean each diluter system.

Those adult and fry tanks receiving the five highest effluent concentrations were continuously aerated with oil-free air to prevent excessively low dissolved oxygen concentrations.

Progeny of fathead minnows (P. promelas) obtained from stock cultures maintained at the Environmental Research Laboratory-Duluth, were used for the life cycle tests. The tests were started by placing fifty 8-12 day old fry in each aquarium and monitoring their survival. The test animals were photographed at 30, 60, 120, and 310 days (adult termination) into the test. The photographs were enlarged to determine the lengths of the surviving test animals.

If the total number of spawnings with at least 50 viable eggs exceeded 10 in a given tank, only eggs from every third spawning were incubated. In addition, when time and spawnings permitted, hatchability information was obtained on eggs produced in dilution water tanks and incubated in high concentration effluent tanks.

If more than 5 of the 50 eggs in any one incubation were unaccounted for, that incubation attempt was discarded. Loss of eggs and/or fry was not uncommon, due to their small size and the nature of the effluent.

Forty 1-2 day old fry from each reproducing adult tank were placed in fry chambers receiving the same concentration of effluent as the adult tank in which they were produced. In an attempt to obtain more complete data on second generation fish, some fry were transferred from the 100 percent dilution water tanks in which they were spawned and incubated to high effluent concentration fry tanks for which no fry were produced in the respective adult tanks. The lengths of all fry were measured photographically at 30 and 60 days. At 60 days the fry were discarded and the tank was then available for restocking.

All fish were fed twice daily, once with a mixture of live brine shrimp nauplii and a commercial trout preparation, and once with live brine shrimp nauplii only. Excess food and other debris were siphoned from the adult test chambers daily. Fry chambers were not cleaned until the fish were 10 days old.

Communication with local industry as well as close surveillance of disinfectant demand enabled the bioassay staff to forecast unusual effluent conditions which might be temporarily lethal to the test fish. In such circumstances, the effluent supply to the test chambers was halted and the fish were maintained with only a supply of dilution water. As soon as the effluent returned to its normal condition (generally less than 1 day) its flow to test chambers was restored.

Dunnett's test[14] was performed on all growth data for all treatment types and concentrations to determine significant ($p = 0.05$) differences between fish reared in effluent and dilution water within a treatment type, and between fish reared in similar concentrations of effluent and dilution water.

RESULTS AND DISCUSSION

The mean residual chemical levels in the head tanks, adult chambers, and fry chambers of the various effluent streams are shown in Tables 6 and 7. Frequent changes in the quality of the Wyoming effluent produced increased variations in the residual chemical levels in all effluent streams.

As in the Grandville study,[8] the Cl_2 residual was the most consistent of those measured. This was partly due to the continuous residual Cl_2 analyzer placed on the chlorinated stream, which enabled operators to adjust dosage frequently to achieve a predetermined residual. Another factor contributing

Table 6 . THE MEAN RESIDUAL CHEMICAL LEVELS (mg/1), SAMPLE SIZES, AND STANDARD DEVIATIONS MEASURED IN HEAD TANKS AND ADULT TEST CHAMBERS DURING THE LIFE CYCLE TESTS

Effluent Stream	Head Tank	Nominal Percent Effluent Concentration						
		50	25	18.75	12.5	6.25	3.12	1.56
Chlorinated								
Chlorine Residual	1.432	0.299	0.085	0.059	0.040	0.018	0.012	0.010
Sample Size	85	6	213	114	114	81	83	81
Standard Deviation	0.619	0.124	0.071	0.050	0.030	0.015	0.010	0.008

Effluent Stream	Head Tank	Nominal Percent Effluent Concentration						
		100	50	25	12.5	6.25	3.12	1.56
Dechlorinated								
Sulfite Residual	1.851	0.010	0.048	0.005	0.002	0.000	0.000	0.000
Sample Size	85	156	140	115	80	84	66	80
Standard Deviation	1.868	0.050	0.140	0.026	0.006	0.001	0.000	0.000
Chlorobrominated								
Bromine Chloride Residual	0.089	0.008	0.008	0.005	0.005	0.010	0.006	0.005
Sample Size	77	6	207	114	104	54	64	79
Standard Deviation	0.093	0.005	0.015	0.005	0.006	0.019	0.006	0.010
Ozonated								
Ozone Residual	0.008	0.003	0.002	0.002	0.002	0.002	0.002	0.001
Sample Size	81	173	119	108	77	75	75	74
Standard Deviation	0.015	0.003	0.002	0.002	0.002	0.002	0.002	0.002

Table 7. THE MEAN RESIDUAL CHEMICAL LEVELS (mg/1), SAMPLE SIZES, AND STANDARD DEVIATIONS MEASURED IN FRY TEST CHAMBERS DURING THE LIFE CYCLE TESTS

	Nominal Percent Effluent Concentration						
Effluent Stream	50	25	18.75	12.5	6.25	3.12	1.56
Chlorinated							
Chlorine Residual	0.055	0.041	0.027	0.021	0.012	0.010	0.006
Sample Size	117	59	56	54	47	49	53
Standard Deviation	0.096	0.085	0.025	0.012	0.012	0.006	0.006

	Nominal Percent Effluent Concentration						
Effluent Stream	100	50	25	12.5	6.25	3.12	1.56
Dechlorinated							
Sulfite Residual	0.215	0.117	0.037	0.000	0.000	0.074	0.001
Sample Size	116	58	50	51	50	51	52
Standard Deviation	0.478	0.266	0.121	0.000	0.002	0.470	0.004
Chlorobrominated							
Bromine Chloride Residual	0.003	0.005	0.003	0.003	0.002	0.002	0.003
Sample Size	113	54	48	52	47	46	47
Standard Deviation	0.009	0.009	0.005	0.008	0.004	0.004	0.004
Ozonated							
Ozone Residual	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Sample Size	115	56	49	49	48	46	46
Standard Deviation	0.002	0.002	0.002	0.001	0.002	0.001	0.002

to the relatively uniform Cl_2 residuals was the reliability of the dosing system which seldom malfunctioned.

The principal reason for the great variation in mean residual sulfite concentrations in the dechlorinated effluent was the lack of capability for the continuous analysis of residual sulfite to assist in the adjustment of SO_2 feed rates as demand varied. A continuous analyzer for residual BrCl placed in the contact chamber corresponding to a 5-minute detention time was used to adjust the dose of BrCl applied to the chlorobrominated effluent stream, but frequent failures of the analyzer or dosing system contributed to the considerable variation of residual BrCl.

Ozone was generally applied at a constant rate, and thus residual O_3 varied considerably with demand. Because of the limited capacity of the O_3 generation-dosing system, it was often impossible to meet the high O_3 demand of the wastewater.

With the exception of residual SO_2, residual chemical concentrations in the fry chambers (Table 7) were lower than those observed in the respective adult chambers. This was partially the result of variations in effluent demand. In addition, some organic matter was allowed to accumulate in fry tanks to promote the growth of microscopic organisms which constitute an important fraction of the diet of young fish. This accumulation of organic matter in fry tanks tended to increase the chemical demand and to decrease measured residuals.

The well water used to dilute the effluent entered its head box with a low DO content and was constantly aerated to an acceptable DO concentration (Table 8). The nondisinfected effluent stream lacked the settling time of a disinfectant contact chamber and carried a greater load of suspended solids to its bioassay laboratory head tank. The consequent accumulation of additional sludge in the head tank increased the oxygen demand and decreased the DO concentration in that tank. The dechlorinated effluent head tank exhibited the lowest mean DO concentration of any disinfected effluent head tank. This lower DO was probably the result of the excess sulfite in this stream reacting with oxygen to form sulfate, as well as the BOD produced in this tank where biological oxidation of sludge was not inhibited by residual disinfectant.

Aeration of the higher effluent concentration fish tanks provided an adequate supply of DO for the test animals. The lowest mean DO was 4.1 mg/l in the 100 percent chlorobrominated fry chamber. This is approximately 50 percent saturation at the 25C test temperature (Table 9) and was probably within the safe limits of DO for *P. promelas*.

Tables 10 and 11 summarize some of the characteristics of the various test streams as measured in the respective head tanks and fish tanks. According to the tables presented in Thurston, *et al.*,[15] the mean total ammonia nitrogen concentrations recorded in the highest concentration fish tanks may have been great enough to produce mortality in the test animals. However, all other characteristics were within the general tolerance limits of fish.[16]

Table 8. THE MEAN DISSOLVED OXYGEN CONCENTRATIONS (mg/1) MEASURED IN HEAD TANKS AND TEST CHAMBERS DURING THE LIFE CYCLE STUDIES

Stream	Head Tank	Test Chambers	Nominal Percent Effluent Concentration							
			100	50	25	12.5	6.25	3.12	1.56	0.00
Dilution Water	4.7[a]	b	b							
Nondisinfected	1.6	Adult	5.4	5.6	5.7	5.7	5.2	5.6	5.8	6.2
		Fry	4.2	5.2	6.1	6.6	6.3	6.4	6.9	7.3
Dechlorinated	3.3	Adult	5.9	5.6	5.9	5.8	5.8	6.1	5.7	6.3
		Fry	5.1	4.6	4.6	6.8	6.1	6.5	7.0	7.3
Chlorobrominated	3.6	Adult	6.1	5.5	5.1	5.4	5.5	5.9	5.7	6.3
		Fry	4.1	4.2	4.4	5.2	6.4	6.2	6.4	7.0
Ozonated	4.5	Adult	5.6	5.8	5.7	6.3	6.0	6.3	5.9	6.5
		Fry	4.5	5.4	6.2	5.5	6.7	6.8	6.6	5.8

Stream	Head Tank	Test Chambers	Nominal Percent Effluent Concentration							
			50	25	18.75	12.5	6.25	3.12	1.56	0.00
Chlorinated	5.1	Adult	4.8	5.5	5.7	6.1	6.3	6.2	5.8	6.2
		Fry	4.9	6.1	6.2	6.3	7.3	6.8	6.6	6.8

[a] The dilution water head tank and all test chambers with 6.25 percent or more effluent were continuously aerated.

[b] Mean dissolved oxygen measurements for 100 percent dilution water tanks are shown under the 0.00 percent effluent column.

Table 9. MEAN WATER TEMPERATURES (°C) MEASURED IN HEAD TANKS AND ADULT TEST CHAMBERS DURING THE LIFE CYCLE STUDIES

Effluent Type	Mean Temperature In Head Tank	Mean Temperature In Aquaria[a]
Nondisinfected	26.2	24.9
Chlorinated	26.3	24.9
Dechlorinated	27.2	24.8
Chlorobrominated	26.7	24.4
Ozonated	25.5	24.8
Dilution Water	26.5	No data available

[a] All temperatures were measured in aquaria containing a 50 percent effluent concentration.

Table 10. CHARACTERISTICS OF THE TEST STREAMS MEASURED IN THE HEAD TANKS

Stream	Alkalinity as $CaCO_3$ (mg/l)	Acidity as $CaCO_3$ (mg/l)	Hardness as $CaCO_3$ (mg/l)	Conductivity (Micromhos/cm)	Total Ammonia Nitrogen (mg/l)
Nondisinfected					
Mean	281	28	279	1091	11.6
Sample Size	43	38	42	34	40
Standard Deviation	37.6	6.0	22.9	136.8	3.11
Chlorinated					
Mean	269	28	281	1101	11.9
Sample Size	42	38	42	34	40
Standard Deviation	34.7	5.8	23.8	133.1	2.67
Dechlorinated					
Mean	260	41	286	1135	11.9
Sample Size	42	36	40	32	40
Standard Deviation	36.7	9.1	19.9	131.7	3.75
Chlorobrominated					
Mean	272	29	281	1090	11.7
Sample Size	42	38	42	34	40
Standard Deviation	34.0	6.7	22.9	130.1	2.28
Ozonated					
Mean	277	58	280	1097	11.3
Sample Size	43	38	42	34	40
Standard Deviation	37.7	154.9	23.0	126.6	3.91
Dilution Water					
Mean	197	25	782	1663	0.6
Sample Size	43	38	42	33	40
Standard Deviation	2.5	4.9	9.9	251.0	1.42

Table 11. CHARACTERISTICS OF THE TEST STREAMS MEASURED IN THE HIGHEST CONCENTRATION ADULT TEST TANKS

Stream	Alkalinity as $CaCO_3$ (mg/1)	Acidity as $CaCO_3$ (mg/1)	Hardness as $CaCO_3$ (mg/1)	Conductivity (Micromhos/cm)	Total Ammonia Nitrogen (mg/1)
Nondisinfected					
Mean	280	18	280	1087	11.0
Sample Size	43	39	42	36	40
Standard Deviation	43.0	6.3	22.8	112.1	3.48
Chlorinated					
Mean	231	23	534	1410	5.6
Sample Size	43	39	42	36	40
Standard Deviation	20.8	6.9	25.7	164.4	2.81
Dechlorinated					
Mean	254	26	284	1105	11.4
Sample Size	43	39	40	34	40
Standard Deviation	37.9	11.5	21.8	128.0	4.11
Chlorobrominated					
Mean	272	20	281	1093	10.7
Sample Size	43	39	42	36	40
Standard Deviation	37.3	7.6	21.7	111.0	3.16
Ozonated					
Mean	273	19	283	1088	9.8
Sample Size	42	39	42	35	40
Standard Deviation	43.3	6.2	22.9	105.2	3.60
Dilution Water					
Mean	197	16	784	1690	0.4
Sample Size	43	39	42	35	39
Standard Deviation	3.3	5.4	12.3	207.7	0.60

The concentrations of selected metals in the nondisinfected effluent were generally typical for a wastewater treatment plant receiving wastes from plating industries (Table 12). Some of the concentrations measured had the potential to be lethal to aquatic life, especially in the 100 percent effluent concentrations. Through cooperation with local industry and vigilance in monitoring chlorine demand, laboratory personnel were generally able to anticipate concentrations of metals which might be toxic to the test animals. When such conditions were expected, effluent was temporarily diverted around the bioassay laboratory until tests indicated that metal concentrations were again safe for the test animals. This procedure prevented mass mortality from metal toxicity during the life cycle study.

The chemical and physical characteristics of respective concentrations of the different effluent streams were generally uniform and safe to the test animals. Thus, differences in results between test animals in disinfected effluent and those in nondisinfected effluent were attributed to the disinfection process.

Mortality

First generation test fish showed high mortality in 100 percent nondisinfected effluent during the first 30 days of the life cycle test (Table 13). The mortality of that period was directly related to relatively high concentrations of heavy metals and cyanide in the effluent. As a result of this high mortality, both 100 percent concentration tanks of nondisinfected effluent were restocked on day 31 with test animals of the same age as the original test animals.

No other first generation mortality with an obvious relationship to effluent concentration occurred in nondisinfected effluent. The mortality observed between days 120 and 240 in the various concentrations of nondisinfected effluent was the result of a disease which was not related to effluent quality. This same disease-related mortality was observed in all test streams.

Second generation test animals exhibited a high mortality, similar to that observed in first generation fish, when exposed to undiluted nondisinfected effluent (Table 14). Thus, it would appear that 100 percent nondisinfected effluent was lethal to young fathead minnows even in the absence of any chemical disinfectant.

First generation test fish in 50 percent chlorinated effluent died within three days when exposed to total residual chlorine concentrations of 0.2 to 0.4 mg/1 (Table 15). These tanks were restocked on day 4, but once again total mortality occurred within 24 hours. First generation fathead minnows in 25 percent chlorinated effluent (mean total residual Cl_2 concentration of 0.165 mg/1) were visibly stressed during the first 30 days of the study and also exhibited reduced survival. No further mortality that could be directly related to chlorinated effluent was observed in any lower test concentration for the remainder of the study. These data are presented in Table 15.

Table 12. MEAN METAL AND CYANIDE CONCENTRATIONS (mg/1) MEASURED IN THE NONDISINFECTED EFFLUENT DURING THE LIFE CYCLE STUDIES[a]

	Chromium	Copper	Nickel	Zinc	Iron	Cyanide
Number of Samples	287	287	287	287	287	136
Mean Concentration	0.084	0.045	0.511	0.149	1.031	0.159
Standard Deviation	0.085	0.036	0.223	0.082	0.865	0.416
Minimum Concentration	0.010	0.000	0.060	0.010	0.070	0.000
Maximum Concentration	0.570	0.480	2.580	0.540	5.640	9.900

[a] Measured by atomic absorption spectrometry

Table 13. NUMBER OF FIRST GENERATION _P. PROMELAS_ SURVIVING IN NONDISINFECTED EFFLUENT

	Nominal Percent Nondisinfected Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100.0
Survival/100 at day 30	97	96	99	94	99	98	99	3
C.I. for prob. of survival thru day 30	0.92–0.99	0.90–0.98	0.95–0.99	0.88–0.97	0.94–0.99	0.93–0.99	0.95–0.99	0.01–0.08
Survival/100 at day 60	97	90	99	93	98	96	94	93[a]
C.I. for prob. of survival thru day 60	0.92–0.99	0.83–0.94	0.95–0.99	0.86–0.97	0.93–0.99	0.90–0.98	0.88–0.97	0.86–0.97
No. of fish kept for further study[b]	30	30	30	30	30	30	30	30
Number of fish alive at: 90 days	29	21	16	30	29	30	29	30
120 days	21	14	15	30	29	30	29	30
150 days	20	14	15	30	17	30	29	22
180 days	16[c]	9[c]	7	25[c]	16	29	29	20[c]
210 days	9	0	7	14	4	29	29	6
240 days	7	0	7	11[c]	4	29	28	0
270 days	7	0	7	11	4	29	24	0
310 days	7	0	7	11	4	28	21	0
C.I. for prob. of survival day 60–310	0.12–0.41	0.00–0.11	0.12–0.41	0.22–0.54	0.05–0.30	0.79–0.98	0.52–0.83	0.00–0.11

[a] Both 100 percent concentration tanks were restocked on day 31

[b] The number of fish in each effluent concentration was reduced to 30 on day 60

[c] Excess males removed from tanks

Table 14. NUMBER OF SECOND GENERATION *P. PROMELAS* SURVIVING IN THE NONDISINFECTED EFFLUENT

	Nominal Percent Nondisinfected Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100.0
30 Days of Age								
Number Surviving/Started	36/40	72/80	50/80	25/40	18/40	22/40	b	0/40
Confidence Interval[a]	0.77-0.96	0.81-0.95	0.52-0.72	0.47-0.76	0.31-0.60	0.40-0.69		0.00-0.08
60 Days of Age								
Number Surviving/Started	36/40	72/80	49/80	23/40	18/40	22/40		
Confidence Interval	0.77-0.96	0.81-0.95	0.50-0.71	0.42-0.71	0.31-0.60	0.40-0.69		

[a] 95 Percent confidence interval for the true probability of survival

[b] No data available

Table 15. NUMBER OF FIRST GENERATION P. PROMELAS SURVIVING IN CHLORINATED EFFLUENT

	Nominal Percent Chlorinated Effluent							
	0.000	1.56	3.12	6.25	12.5	18.75	25.0	50.0
Survival/100 at day 30	80 (0.002)[a]	95 (0.017)	38 (0.020)	100 (0.026)	76 (0.059)	90 (0.122)	46 (0.165)	0 (0.299)
C.I.[b] for prob. of survival thru day 30	0.71–0.87	0.89–0.98	0.29–0.48	0.96–1.00	0.67–0.83	0.83–0.94	0.37–0.56	0.00–0.03
Survival/100 at day 60	79 (0.003)	95 (0.016)	38 (0.021)	99 (0.032)	75 (0.065)	85 (0.109)	46 (0.161)	0
C.I. for prob. of survival thru day 60	0.70–0.86	0.89–0.98	0.29–0.48	0.95–0.99	0.66–0.82	0.77–0.91	0.37–0.56	0.00–0.03
No. of fish kept for further study[c]	30	30	30	30	30	30	27	0
Number of fish alive at: 90 days	30 (0.003)	30 (0.015)	30 (0.019)	30 (0.033)	30 (0.062)	30 (0.098)	27 (0.151)	0
120 days	29 (0.003)	29 (0.014)	29 (0.019)	30 (0.031)	30 (0.056)	30 (0.085)	27 (0.128)	0
150 days	29 (0.003)	29 (0.014)	29 (0.018)	30 (0.029)	30 (0.053)	30 (0.081)	26 (0.115)	0
180 days	27[d] (0.003)	22[d] (0.014)	22[d] (0.018)	20[d] (0.028)	25[d] (0.051)	27[d] (0.077)	25 (0.110)	
210 days	27 (0.003)	22 (0.013)	22 (0.017)	20 (0.026)	25 (0.049)	27 (0.073)	24 (0.104)	0
240 days	27 (0.003)	18 (0.012)	22 (0.016)	20 (0.024)	23[d] (0.047)	27 (0.070)	24 (0.100)	0
270 days	27 (0.002)	17 (0.011)	22 (0.014)	20 (0.021)	23 (0.043)	26 (0.064)	24 (0.093)	0
310 days	27 (0.002)	16 (0.009)	22 (0.012)	20 (0.018)	23 (0.040)	25 (0.059)	24 (0.085)	0
C.I. for prob. of survival day 60–310	0.74–0.97	0.36–0.70	0.56–0.86	0.49–0.81	0.59–0.88	0.66–0.93	0.63–0.90	0.00–0.11

[a] Mean total residual chlorine (mg/l)

[b] 95 percent confidence interval for the true probability of survival

[c] The number of fish in each effluent concentration was reduced to a maximum of 30 on day 60

[d] Excess males removed from tanks

First generation test fish in this study exhibited a greater tolerance to chlorinated effluent than their counterparts in the Grandville study. For example, a 53 day mean total residual Cl_2 concentration of 0.076 mg/l in the 20 percent effluent concentration resulted in a 0.34-0.53 probability of survival at Grandville (Table 22, Vol. 1),[8] while a 60 day mean total residual Cl_2 concentration more than two times as great (0.161 mg/l) in the 25 percent effluent concentration resulted in about the same probability of survival (0.37-0.56) at Wyoming. While the cause of this difference in tolerance is unknown, it may be related to natural variations in test replication, differences in the qualities of the raw wastewaters at the two plants, differences between activated sludge and trickling filter effluents, or some other factor(s).

Second generation test animals were exposed to low residual Cl_2 levels and showed no mortality related to residual Cl_2 in the effluent (Table 16). While total mortality of test animals did occur in the 50 percent effluent concentration, the mean residual Cl_2 present in that effluent concentration was 0.008 mg/l, far less than the threshold value for lethality (0.045 mg/l) that was observed in the Grandville study.[8] Further, the total mortality of second generation fish in the 50 percent concentrations of all disinfected effluent streams supported the conclusion that mortality did not result from the low levels of residual Cl_2 to which second generation test animals were exposed.

First generation test fish exposed to dechlorinated effluent survived much better than those which were exposed to chlorinated or nondisinfected effluent (Table 17). Dechlorination eliminated the toxicity of chlorinated effluent to young fathead minnows. The improved survival over fish reared in respective concentrations of nondisinfected effluent might be the result of the greater aeration (and subsequent higher DO levels) of the dechlorinated stream (see Chapter III). The mortality observed in the 100 percent concentration of dechlorinated effluent after day 150 was the result of a disease outbreak in the two undiluted effluent test chambers. The limitation of the spread of the disease to those two tanks was probably the result of stress on those test animals in the undiluted effluent at a time when effluent quality was very low (see Chapter III).

Second generation test fish did not survive in either 50 percent or 100 percent dechlorinated effluent (Table 18). However, since a similar pattern of survival was also observed in second generation fish in nondisinfected effluent (Table 14), the mortality in the 50 and 100 percent concentrations of dechlorinated effluent was probably the result of poor effluent quality rather than the chlorination-dechlorination process.

In the chlorobrominated effluent stream, only the first generation fish exposed to 100 percent effluent exhibited unusually low survival (Table 19). This mortality appeared to result from low effluent quality rather than the disinfection process, because similar mortality was observed in the nondisinfected effluent stream (Table 13) and because only very low (<0.017 mg/l) BrCl residuals were present during the first 30 days of the test. Likewise, the survival pattern of second generation test fish (Table 20) was similar to that of second generation test animals reared in nondisinfected effluent,

Table 16. NUMBER OF SECOND GENERATION P. PROMELAS SURVIVING IN THE CHLORINATED EFFLUENT

	Nominal Percent Chlorinated Effluent							
	0.00	1.56	3.12	6.25	12.5	18.75	25.0	50.0
30 Days of Age								
Number Surviving/Started	52/80	15/40	a	a	54/80	38/80	9/40	0/40
Confidence Interval[b]	0.54–0.75	0.24–0.52			0.57–0.77	0.37–0.58	0.12–0.38	0.00–0.08
$\bar{x}$ Residual, mg/1[c]	0.000	0.000			0.007	0.003	0.005	0.008
60 Days of Age								
Number Surviving/Started	52/80	15/40			49/80	37/80	9/40	
Confidence Interval	0.54–0.75	0.24–0.53			0.50–0.71	0.36–0.57	0.12–0.38	
$\bar{x}$ Residual, mg/1	0.000	0.002			0.001	0.009	0.021	

[a] No data available

[b] 95 Percent confidence interval for the true probability of survival

[c] Mean total residual chlorine (mg/1)

Table 17. NUMBER OF FIRST GENERATION *P. PROMELAS* SURVIVING IN DECHLORINATED EFFLUENT

	Nominal Percent Dechlorinated Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100
Survival/100 at day 30	75 (0.000)[a]	93 (0.000)	96 (0.000)	99 (0.001)	74 (0.000)	94 (0.000)	96 (0.000)	47 (0.019)
C.I.[b] for prob. of survival thru day 30	0.66–0.82	0.86–0.97	0.90–0.98	0.95–0.99	0.65–0.82	0.88–0.97	0.90–0.98	0.38–0.57
Survival/100 at day 60	75 (0.000)	91 (0.000)	96 (0.000)	98 (0.000)	63 (0.003)	90 (0.007)	94 (0.010)	47 (0.016)
C.I. for prob. of survival thru day 60	0.66–0.82	0.84–0.95	0.90–0.98	0.93–0.99	0.53–0.72	0.83–0.94	0.88–0.97	0.38–0.57
No. of fish kept for further study[c]	30	30	30	30	30	30	30	30
Number of fish alive at: 90 days	30 (0.000)	30 (0.000)	30 (0.000)	30 (0.000)	30 (0.002)	30 (0.006)	30 (0.012)	30 (0.012)
120 days	30 (0.000)	30 (0.000)	30 (0.000)	29 (0.000)	30 (0.002)	30 (0.005)	30 (0.009)	30 (0.009)
150 days	30 (0.000)	30 (0.000)	30 (0.000)	29 (0.000)	30 (0.001)	30 (0.004)	30 (0.008)	30 (0.009)
180 days	25[d] (0.000)	22[d] (0.000)	24[d] (0.000)	26[d] (0.000)	28[d] (0.001)	26[d] (0.004)	30 (0.007)	0 (0.010)
210 days	25 (0.000)	22 (0.000)	24 (0.000)	26 (0.000)	28 (0.002)	26 (0.003)	30 (0.013)	0
240 days	25 (0.000)	22 (0.000)	24 (0.000)	26 (0.000)	28 (0.002)	26 (0.006)	30 (0.039)	0
270 days	25 (0.000)	22 (0.000)	24 (0.000)	26 (0.000)	28 (0.002)	26 (0.005)	28 (0.051)	0
310 days	25 (0.000)	22 (0.000)	23 (0.000)	26 (0.000)	26 (0.001)	25 (0.005)	28 (0.048)	0
C.I. for prob. of survival day 60–310	0.66–0.93	0.56–0.86	0.59–0.88	0.70–0.95	0.70–0.95	0.66–0.93	0.79–0.98	0.00–0.11

[a] Mean residual sulfur dioxide as sulfite (mg/l)

[b] 95 percent confidence interval for the true probability of survival

[c] The number of fish in each effluent concentration was reduced to 30 on day 60

[d] Excess males removed from tanks

Table 18. NUMBER OF SECOND GENERATION *P. PROMELAS* SURVIVING IN THE DECHLORINATED EFFLUENT

	Nominal Percent Dechlorinated Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100.0
30 Days of Age								
Number Surviving/Started	27/40	31/40	63/80	31/80	a	21/40	0/40	0/40
Confidence Interval[b]	0.52-0.80	0.62-0.88	0.69-0.86	0.29-0.50		0.37-0.67	0.00-0.08	0.00-0.08
$\bar{x}$ Residual, mg/1[c]	0.000	0.000	0.000	0.000		0.135	0.388	0.616
60 Days of Age								
Number Surviving/Started	27/40	31/40	51/80	31/80		21/40		
Confidence Interval	0.52-0.82	0.62-0.88	0.53-0.73	0.29-0.50		0.37-0.67		
$\bar{x}$ Residual, mg/1	0.000	0.009	0.000	0.000		0.000		

[a] No data available

[b] 95 Percent confidence interval for the true probability of survival

[c] Mean residual sulfite (mg/1)

Table 19. NUMBER OF FIRST GENERATION P. PROMELAS SURVIVING IN CHLOROBROMINATED EFFLUENT

	Nominal Percent Chlorobrominated Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100.0
Survival/100 at day 30	75 (0.008)[a]	88 (0.005)	62 (0.006)	90 (0.006)	75 (0.007)	88 (0.009)	96 (0.009)	0 (0.009)
C.I.[b] for prob. of survival thru day 30	0.66-0.82	0.80-0.93	0.52-0.71	0.83-0.94	0.66-0.82	0.80-0.93	0.90-0.98	0.00-0.03
Survival/100 at day 60	75 (0.006)	89 (0.011)	61 (0.007)	90 (0.014)	75 (0.009)	81 (0.009)	87 (0.015)	0
C.I. for prob. of survival thru day 60	0.66-0.82	0.81-0.94	0.51-0.70	0.83-0.94	0.66-0.82	0.72-0.87	0.79-0.92	0.00-0.03
No. of fish kept for further study[c]	30	30	30	30	30	30	30	0
Number of fish alive at: 90 days	30 (0.006)	30 (0.010)	30 (0.006)	20 (0.010)	28 (0.008)	29 (0.009)	22 (0.013)	0
120 days	16 (0.006)	30 (0.010)	30 (0.007)	15 (0.011)	24 (0.007)	29 (0.008)	22 (0.011)	0
150 days	16 (0.005)	30 (0.009)	30 (0.006)	15 (0.010)	24 (0.007)	29 (0.008)	22 (0.010)	0
180 days	11[d] (0.005)	26[d] (0.009)	22[d] (0.006)	12[d] (0.010)	21[d] (0.007)	28[d] (0.007)	21 (0.010)	0
210 days	9 (0.004)	26 (0.008)	10 (0.006)	12 (0.010)	16 (0.007)	28 (0.007)	21 (0.009)	0
240 days	9 (0.004)	26 (0.007)	10 (0.006)	12 (0.010)	16 (0.007)	26 (0.007)	15 (0.009)	0
270 days	9 (0.003)	26 (0.006)	10 (0.006)	12 (0.009)	15 (0.006)	26 (0.006)	14 (0.009)	0
310 days	8 (0.003)	11 (0.005)	10 (0.005)	12 (0.007)	14 (0.006)	26 (0.005)	11 (0.008)	0
C.I. for prob. of survival day 60-310	0.14-0.44	0.22-0.54	0.19-0.51	0.25-0.58	0.30-0.64	0.70-0.95	0.22-0.54	0.00-0.11

[a] Mean total residual bromine chloride (mg/l)

[b] 95 percent confidence interval for the true probability of survival

[c] The number of fish in each effluent concentration was reduced to 30 on day 60

[d] Excess males removed from tanks

Table 20. NUMBER OF SECOND GENERATION P. PROMELAS SURVIVING IN THE CHLOROBROMINATED EFFLUENT

	Nominal Percent Chlorobrominated Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100.0
30 Days of Age								
Number Surviving/Started	56/80	55/80	34/80	16/40	58/80	37/80	0/40	a
Confidence Interval[b]	0.59–0.79	0.58–0.78	0.32–0.53	0.26–0.55	0.62–0.81	0.36–0.57	0.00–0.08	
$\bar{x}$ Residual, mg/1[c]	0.000	0.002	0.001	0.000	0.000	0.001	0.000	
60 Days of Age								
Number Surviving/Started	30/80	55/80	34/80	15/40	29/80	15/80		
Confidence Interval	0.28–0.48	0.58–0.79	0.32–0.53	0.24–0.53	0.27–0.47	0.12–0.29		
$\bar{x}$ Residual, mg/1	0.000	0.002	0.001	0.000	0.001	0.000		

[a] No data available

[b] 95 Percent confidence interval for the true probability of survival

[c] Mean total residual bromine chloride (mg/1)

suggesting that the survival of fathead minnows was not affected by the levels of residual BrCl to which they were exposed in this study.

First generation test animals in undiluted ozonated effluent exhibited mortality only as the result of disease and aeration failures. Thus, first generation survival in ozonated effluent was superior to that in any other effluent stream (Table 21). The only other time that the survival of first generation fathead minnows approached the survival level of subjects reared in 100 percent ozonated effluent was in dechlorinated effluent (Table 17). Since both of these effluent streams were subjected to greater mixing and aeration during the treatment process, the improved survival that we observed may have been related to the DO content of these effluent streams. The survival of second generation test fish in ozonated effluent was poor (Table 22), probably because of the poor quality of the effluent during the period when those fish were cultured.

As in the Grandville study,[8] undiluted nondisinfected effluent was lethal to test subjects. Further, no life cycle mortality was attributed to residual O_3 and, in fact, ozonated effluent produced improved survival of young fathead minnows in 100 percent effluent concentrations. Unlike the Grandville findings, no evidence of death from residual BrCl was observed in the life cycle study, presumably because of the very low BrCl residuals to which the Wyoming test animals were subjected.

The fathead minnow test subjects were more tolerant of residual Cl_2 in the Wyoming study than in the Grandville study. For example, test animals of less than 60 days of age survived mean residual Cl_2 concentrations as high as 0.109 mg/l in 18.75 percent effluent at Wyoming, while mean residual Cl_2 concentrations as low as 0.045 mg/l in 14 percent effluent were lethal in comparable tests at Grandville. The cause of this difference in tolerance at the two test sites is unknown, and, because of the many variables potentially involved, any attempt at an explanation would be pure speculation. Factors which may have affected tolerance levels include: (1) experience and skill of laboratory personnel (greater at Wyoming than Grandville); (2) the characteristics of the raw wastewater stream (Grandville was primarily domestic wastewater, while Wyoming included a large fraction of industrial wastes); (3) differences in wastewater treatment processes (activated sludge at Grandville, trickling filter at Wyoming); and (4) effluent quality at the most sensitive life stage of the test animals (the quality of the Grandville effluent was lowest at the beginning of the study and gradually improved thereafter). Finally, it is possible that the observed difference is not a real difference, but merely the normal variation characteristic of any replicated test. The significance of the difference in sensitivity to residual Cl_2 observed at the two test sites can only be determined through additional study of the phenomenon.

One of the more definitive effects on mortality in the life cycle tests was observed in the dechlorinated effluent stream. Both the Grandville and Wyoming data illustrate the effectiveness of SO_2 in eliminating the lethal effects of chlorinated effluent concentrations as great as 100 percent. Further, during the course of this study, the dosing of SO_2 proved to be an uncomplicated and reliable process. This suggested that dechlorination with

Table 21. NUMBER OF FIRST GENERATION *P. PROMELAS* SURVIVING IN OZONATED EFFLUENT

	Nominal Percent Ozonated Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100.0
Survival/100 at day 30	96 (0.002)[a]	97 (0.002)	95 (0.003)	100 (0.003)	98 (0.003)	95 (0.003)	96 (0.004)	91 (0.004)
C.I.[b] for prob. of survival thru day 30	0.90–0.98	0.92–0.99	0.89–0.98	0.96–1.00	0.93–0.99	0.89–0.98	0.90–0.98	0.84–0.95
Survival/100 at day 60	96 (0.002)	94 (0.002)	92 (0.003)	95 (0.003)	95 (0.003)	93 (0.003)	91 (0.004)	84 (0.004)
C.I. for prob. of survival thru day 60	0.90–0.98	0.88–0.97	0.85–0.96	0.89–0.98	0.89–0.98	0.86–0.97	0.84–0.95	0.76–0.90
No. of fish kept for further study[c]	30	30	30	30	30	30	30	30
Number of fish alive at: 90 days	30 (0.002)	30 (0.003)	30 (0.003)	28 (0.002)	29 (0.003)	30 (0.003)	30 (0.003)	30 (0.005)
120 days	30 (0.002)	30 (0.003)	30 (0.003)	28 (0.003)	29 (0.003)	30 (0.003)	30 (0.003)	28 (0.004)
150 days	30 (0.002)	30 (0.003)	30 (0.003)	28 (0.003)	29 (0.003)	30 (0.002)	30 (0.003)	27 (0.004)
180 days	26[d] (0.002)	22[d] (0.003)	26[d] (0.002)	18[d] (0.003)	22[d] (0.002)	24[d] (0.002)	22[d] (0.003)	25[d] (0.004)
210 days	26 (0.001)	20 (0.003)	26 (0.002)	18 (0.002)	22 (0.002)	23 (0.002)	22 (0.003)	22 (0.003)
240 days	26 (0.001)	20 (0.002)	26 (0.002)	18 (0.003)	22 (0.002)	22 (0.002)	22 (0.003)	0
270 days	25 (0.001)	15 (0.002)	26 (0.002)	18 (0.002)	21 (0.002)	22 (0.002)	18 (0.003)	0
310 days	25 (0.001)	15 (0.002)	26 (0.002)	18 (0.002)	20 (0.002)	22 (0.002)	15 (0.002)	0
C.I. for prob. of survival day 60–310	0.66–0.93	0.33–0.67	0.70–0.95	0.42–0.75	0.49–0.81	0.56–0.86	0.33–0.67	0.00–0.11

[a] Mean residual ozone (mg/l)

[b] 95 percent confidence interval for the true probability of survival

[c] The number of fish in each effluent concentration was reduced to 30 on day 60

[d] Excess males removed from tanks

Table 22. NUMBER OF SECOND GENERATION P. PROMELAS SURVIVING IN THE OZONATED EFFLUENT

	Nominal Percent Ozonated Effluent							
	0.00	1.56	3.12	6.25	12.5	25.0	50.0	100.0
30 Days of Age								
Number Surviving/Started	49/80	43/80	49/80	31/80	0/40	a	0/40	0/40
Confidence Interval[b]	0.50-0.71	0.43-0.64	0.50-0.71	0.29-0.50	0.00-0.08		0.00-0.08	0.00-0.08
$\bar{x}$ Residual, mg/1[c]	0.000	0.001	0.001	0.000	0.000		0.000	0.001
60 Days of Age								
Number Surviving/Started	49/80	43/80	49/80	31/80				
Confidence Interval	0.50-0.71	0.43-0.64	0.50-0.71	0.29-0.50				
$\bar{x}$ Residual, mg/1	0.000	0.001	0.002	0.000				

[a] No data available

[b] 95 Percent confidence interval for the true probability of survival

[c] Mean residual ozone (mg/1)

SO_2 has the potential to be an effective and relatively simple method of preventing any long-term effects which might be associated with the Cl_2 residual in chlorinated effluent.

Growth

Tables 23-28 summarize the mean length data for first and second generation test fish. The data were statistically analyzed with Dunnett's test in which the mean length of the fish in one concentration of a disinfected effluent is compared with the mean length of fish in the respective concentration of nondisinfected effluent, and with the mean length of the fish in the dilution water control tank on the same test table. Note that this analysis does not compare data from respective effluent concentrations of the other disinfected effluents. For example, the mean length of fish in the 25 percent concentration of ozonated effluent is statistically tested against the mean length of fish in 25 percent nondisinfected effluent and the mean length of fish in dilution water on the ozonated effluent test table, but is not statistically tested against the mean lengths of fish reared in the 25 percent concentrations of the other disinfected streams (chlorinated, dechlorinated, and chlorobrominated).

Significant differences were observed in the mean lengths of first and second generation test animals reared in dilution water (Tables 23, 27, and 28), in spite of the fact that the ecosystems within these tanks were so planned and maintained as to be similar in all measured characteristics.

Uncontrolled variables which might have affected growth in the dilution water control tanks included population size, the sex composition of a population, and the development of natural populations of planktonic food organisms within a test tank.

Inconsistent differences in mean length data were also observed between fish reared in different concentrations of the same effluent stream and in their dilution water controls. These inconsistencies generally made it impossible to identify trends in growth related to effluent concentration.

The inconsistencies mentioned above greatly limit the conclusions which can be drawn from the growth data. Further, Dunnett's test applied to the mean weights of fish in each concentration of each test stream at the termination of the life cycle study failed to show relationships between effluent type or concentration and body weight. Thus, the statistical analyses of the available data did not demonstrate that any of the effluent treatments tested had any effect on the growth of fathead minnows. However, the growth of life cycle test fish in the higher concentrations of ozonated effluent, because of its consistency and pattern, does deserve additional discussion.

As seen in Tables 23-26, the mean lengths of fish reared in 50 and 100 percent concentrations of ozonated effluent tended to be greater than their counterparts in any other effluent stream. While these length differences were not always statistically significant, the consistency of the trend was such as to suggest that fathead minnows achieved greater mean lengths in high concentrations of ozonated effluent than in similar high concentrations

Table 23. MEAN LENGTHS (IN mm) OF FIRST GENERATION P. PROMELAS ON DAY 30 OF THE LIFE CYCLE TEST

Nominal Effluent Concetration and Data		Effluent Stream				
		Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	97	80	75	74	96
	x̄	22.7	20.9	20.2	20.6	19.6
	S.D.	2.82	3.24	2.75	2.62	2.69
	SDND[a]		Yes	Yes	Yes	Yes
1.56%	N	96	95	93	88	97
	x̄	21.2	22.1	20.7	20.0	20.3
	S.D.	3.45	2.69	3.29	3.44	3.09
	SDDW[b]	Yes	Yes	No	No	No
	SDND		No	No	Yes	No
	x̄ Residual		0.017	0.000	0.005	0.002
3.12%	N	98	38	96	62	95
	x̄	23.4	25.5	20.9	21.3	20.4
	S.D.	3.18	2.93	3.06	3.10	3.09
	SDDW	No	Yes	No	No	No
	SDND		Yes	Yes	Yes	Yes
	x̄ Residual		0.020	0.000	0.006	0.003
6.25%	N	94	100	99	90	100
	x̄	22.1	21.5	20.4	20.4	20.6
	S.D.	2.80	2.35	2.24	3.02	2.95
	SDDW	No	No	No	No	No
	SDND		No	Yes	Yes	Yes
	x̄ Residual		0.026	0.000	0.006	0.003
12.50%	N	99	76	74	75	98
	x̄	22.4	21.1	20.0	18.4	20.6
	S.D.	2.40	2.35	2.18	3.55	2.92
	SDDW	No	No	No	Yes	No
	SDND		Yes	Yes	Yes	Yes
	x̄ Residual		0.059	0.000	0.007	0.003
25.00%	N	98	46	94	88	95
	x̄	22.0	17.2	21.0	20.4	20.9
	S.D.	3.27	2.42	2.19	2.12	2.99
	SDDW	No	Yes	No	No	Yes
	SDND		Yes	No	Yes	Yes
	x̄ Residual		0.165	0.000	0.009	0.003
50.00%	N	99	N.D.[c]	96	96	96
	x̄	21.2		18.9	19.7	21.5
	S.D.	2.73		2.30	2.36	3.22
	SDDW	Yes		Yes	No	Yes
	SDND			Yes	Yes	No
	x̄ Residual			0.000	0.009	0.004
100.00%	N	60	N.D.	47	N.D.	91
	x̄	14.5		14.8		19.1
	S.D.	1.67		2.01		2.05
	SDDW	Yes		Yes		No
	SDND			No		Yes
	x̄ Residual			0.019		0.004

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[a] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in the same concentration in the nondisinfected effluent stream.

[b] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in dilution water tanks of the respective effluent stream.

[c] No data available.

Table 24. MEAN LENGTHS (IN mm) OF FIRST GENERATION P. PROMELAS On DAY 60 OF THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream				
		Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	97	79	75	75	96
	x̄	32.7	31.9	32.0	32.2	31.1
	S.D.	3.14	4.09	4.92	4.08	3.78
	SDND[a]		No	No	No	No
1.56%	N	90	95	91	89	94
	x̄	32.9	32.4	32.5	31.8	31.5
	S.D.	3.21	3.59	4.33	3.78	4.17
	SDDW[b]	No	No	No	No	No
	SDND		No	No	No	No
	x̄ Residual		0.016	0.000	0.011	0.002
3.12%	N	99	38	96	61	92
	x̄	33.1	38.5	31.8	34.7	32.2
	S.D.	3.50	4.44	4.62	4.30	4.40
	SDDW	No	Yes	No	Yes	No
	SDND		Yes	No	No	No
	x̄ Residual		0.021	0.000	0.007	0.003
6.25%	N	93	99	98	90	95
	x̄	31.7	31.9	30.7	32.7	32.0
	S.D.	2.97	3.27	3.40	5.16	4.35
	SDDW	No	No	No	No	No
	SDND		No	No	No	No
	x̄ Residual		0.032	0.000	0.014	0.003
12.50%	N	98	75	63	75	95
	x̄	30.8	32.8	32.3	33.4	31.4
	S.D.	2.41	3.01	3.53	3.85	4.05
	SDDW	Yes	Yes	No	No	No
	SDND		Yes	Yes	Yes	No
	x̄ Residual		0.065	0.003	0.009	0.003
25.00%	N	96	46	90	81	93
	x̄	31.7	28.8	32.8	31.0	33.3
	S.D.	2.64	2.92	2.94	3.17	4.10
	SDDW	No	Yes	No	No	Yes
	SDND		Yes	No	No	Yes
	x̄ Residual		0.161	0.007	0.009	0.003
50.00%	N	94	N.D.[c]	94	87	91
	x̄	31.9		31.9	30.6	34.9
	S.D.	2.73		2.70	3.82	4.13
	SDDW	No		No	No	Yes
	SDND			No	Yes	Yes
	x̄ Residual			0.010	0.015	0.004
100.00%	N	93	N.D.	47	N.D.	84
	x̄	27.3		24.2		33.6
	S.D.	5.31		4.19		3.27
	SDDW	Yes		Yes		Yes
	SDND			Yes		Yes
	x̄ Residual			0.016		0.004

[a] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in the same concentration in the nondisinfected effluent stream.

[b] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in dilution water tanks of the respective effluent stream.

[c] No data available.

Table 25. MEAN LENGTHS (IN mm) OF FIRST GENERATION P. PROMELAS ON DAY 120 OF THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream				
		Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	23	29	30	16	30
	$\bar{x}$	51.1	48.7	50.7	51.3	48.5
	S.D.	6.70	5.29	5.83	4.63	5.19
	SDND[a]		No	No	No	No
1.56%	N	14	29	30	29	30
	$\bar{x}$	53.8	52.9	52.8	51.3	52.0
	S.D.	7.28	5.15	6.29	5.12	5.99
	SDDW[b]	No	Yes	No	No	No
	SDND		No	No	No	No
	$\bar{x}$ Residual		0.014	0.000	0.010	0.003
3.12%	N	15	29	30	30	30
	$\bar{x}$	54.4	55.0	51.5	52.5	51.0
	S.D.	4.68	7.65	5.79	7.27	6.14
	SDDW	No	Yes	No	No	No
	SDND		No	No	No	No
	$\bar{x}$ Residual		0.018	0.000	0.007	0.002
6.25%	N	30	30	30	15	28
	$\bar{x}$	51.8	54.2	50.2	51.8	55.7
	S.D.	5.67	4.89	5.22	6.12	6.98
	SDDW	No	Yes	No	No	Yes
	SDND		No	No	No	Yes
	$\bar{x}$ Residual		0.031	0.000	0.010	0.003
12.50%	N	29	30	30	24	29
	$\bar{x}$	49.8	53.8	51.5	50.2	54.5
	S.D.	5.32	5.73	5.51	6.50	6.90
	SDDW	No	Yes	No	No	Yes
	SDND		Yes	No	No	Yes
	$\bar{x}$ Residual		0.056	0.002	0.007	0.002
25.00%	N	30	27	30	29	30
	$\bar{x}$	47.4	48.6	51.1	49.5	54.9
	S.D.	4.26	5.55	4.65	4.03	6.89
	SDDW	No	No	No	No	Yes
	SDND		No	Yes	No	Yes
	$\bar{x}$ Residual		0.128	0.005	0.008	0.003
50.00%	N	29	N.D.[c]	30	17	30
	$\bar{x}$	45.0		46.1	45.7	55.1
	S.D	3.07		3.48	2.63	5.90
	SDDW	Yes		Yes	Yes	Yes
	SDND			No	No	Yes
	$\bar{x}$ Residual			0.010	0.011	0.003
100.00%	N	30	N.D.	30	N.D.	28
	$\bar{x}$	29.9		29.4		49.3
	S.D.	2.38		3.27		4.16
	SDDW	Yes		Yes		No
	SDND			No		Yes
	$\bar{x}$ Residual			0.009		0.004

[a] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in the same concentration in the nondisinfected effluent stream.

[b] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in dilution water tanks of the respective effluent stream.

[c] No data available.

Table 26. MEAN LENGTHS (IN mm) OF FIRST GENERATION P. PROMELAS AT DAY 310 (TERMINATION) OF THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream				
		Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	7	27	25	8	25
	$\bar{x}$	66.7	64.9	65.0	68.6	65.7
	S.D.	8.44	9.39	9.02	10.51	9.20
	SDND[a]		No	No	No	No
1.56%	N		16	22	11	15
	$\bar{x}$		67.4	68.5	66.2	63.2
	S.D	N.D.[c]	8.07	9.72	7.85	5.58
	SDDW[b]		No	No	No	No
	SDND					
	$\bar{x}$ Residual		0.009	0.000	0.005	0.002
3.12%	N	7	22	23	10	26
	$\bar{x}$	74.0	68.1	67.4	65.4	64.3
	S.D.	3.61	10.55	9.56	8.01	5.83
	SDDW	Yes	No	No	No	No
	SDND		No	No	No	Yes
	$\bar{x}$ Residual		0.012	0.000	0.005	0.002
6.25%	N	11	20	26	12	18
	$\bar{x}$	66.9	68.9	65.2	66.0	68.8
	S.D.	9.73	9.62	7.01	9.69	10.45
	SDDW	No	No	No	No	No
	SDND		No	No	No	No
	$\bar{x}$ Residual		0.018	0.000	0.007	0.002
12.50%	N	4	23	12	14	20
	$\bar{x}$	67.8	66.7	65.8	70.5	66.7
	S.D.	10.75	9.84	7.87	10.59	8.73
	SDDW	No	No	No	No	No
	SDND		No	No	No	No
	$\bar{x}$ Residual		0.040	0.001	0.006	0.002
25.00%	N	28	24	25	26	22
	$\bar{x}$	61.3	63.1	65.8	65.2	65.0
	S.D.	4.58	5.92	6.52	6.67	8.49
	SDDW	No	No	No	No	No
	SDND		No	No	No	No
	$\bar{x}$ Residual		0.085	0.005	0.005	0.002
50.00%	N	21		28	11	15
	$\bar{x}$	53.7		54.5	55.0	63.3
	S.D.	2.74	N.D.	3.33	2.38	6.14
	SDDW	Yes		Yes	Yes	No
	SDND			No	No	Yes
	$\bar{x}$ Residual			0.048	0.008	0.002
100.00%	N					
	$\bar{x}$					
	S.D.	N.D	N.D	N.D	N.D	N.D.
	SDDW					
	SDNS					
	$\bar{x}$ Residual					

[a] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in the same concentration in the nondisinfected effluent stream.

[b] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in dilution water tanks of the respective effluent stream.

[c] No data available.

Table 27. MEAN LENGTHS (IN mm) OF 30 DAY OLD SECOND GENERATION *P. PROMELAS* IN THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream				
		Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	36	52	27	56	49
	$\bar{x}$	24.4	19.6	19.8	21.6	20.9
	S.D.	2.71	3.16	3.44	2.84	2.62
	SDND[a]		Yes	Yes	Yes	Yes
1.56%	N	68	15	31	55	43
	$\bar{x}$	24.3	21.0	22.4	20.8	19.2
	S.D.	2.17	1.49	0.92	2.75	1.80
	SDDW[b]	No	No	Yes	No	Yes
	SDND		Yes	Yes	Yes	Yes
	$\bar{x}$ Residual		0.000	0.000	0.002	0.001
3.12%	N	50		63	34	49
	$\bar{x}$	23.2		22.3	22.9	23.4
	S.D.	1.32	N.D.[c]	2.41	1.68	4.18
	SDDW	No		Yes	No	Yes
	SDND			No	No	No
	$\bar{x}$ Residual			0.000	0.001	0.001
6.25%	N	25		31	16	31
	$\bar{x}$	24.4		21.2	20.7	23.4
	S.D.	3.36	N.D.	3.23	0.98	1.52
	SDDW	No		No	No	Yes
	SDND			Yes	Yes	No
	$\bar{x}$ Residual			0.000	0.000	0.000
12.50%	N	18	54		58	
	$\bar{x}$	19.4	22.5		22.0	
	S.D.	1.11	1.61	N.D.	2.61	N.D.
	SDDW	Yes	Yes		No	
	SDND		Yes		Yes	
	$\bar{x}$ Residual		0.007		0.000	
25.00%	N	22	9	21	37	
	$\bar{x}$	21.7	19.4	15.5	17.5	
	S.D.	1.20	1.39	1.32	1.03	N.D.
	SDDW	Yes	No	Yes	Yes	
	SDND		Yes	Yes	Yes	
	$\bar{x}$ Residual		0.003	0.135	0.001	
50.00%	N					
	$\bar{x}$					
	S.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	SDDN					
	SDND					
	$\bar{x}$ Residual					
100.00%	N					
	$\bar{x}$					
	S.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	SDDW					
	SDND					
	$\bar{x}$ Residual					

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[a] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in the same concentration in the nondisinfected effluent stream.

[b] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in dilution water tanks of the respective effluent stream.

[c] No data available.

Table 28. MEAN LENGTHS (IN mm) OF 60 DAY OLD SECOND GENERATION *P. PROMELAS* IN THE LIFE CYCLE TEST

Nominal Effluent Concentration and Data		Effluent Stream				
		Nondis. (A)	Chlor. (B)	Dechlor. (C)	Chlorobr. (D)	Ozon. (E)
Dilution Water	N	36	52	27	30	49
	$\bar{x}$	38.5	33.3	34.2	34.2	33.9
	S.D.	3.33	2.77	3.09	3.44	3.53
	SDND[a]		Yes	Yes	Yes	Yes
1.56%	N	72	15	31	33	43
	$\bar{x}$	37.3	38.2	37.1	36.5	34.8
	S.D.	3.91	3.05	1.99	2.91	3.57
	SDDW[b]	No	Yes	Yes	Yes	No
	SDND		No	No	No	Yes
	$\bar{x}$ Residual		0.002	0.009	0.002	0.001
3.12%	N	49	N.D.[c]	51	29	49
	$\bar{x}$	39.4		34.2	37.7	37.1
	S.D.	3.62		3.23	2.65	4.88
	SDDW	No		No	Yes	Yes
	SDND			Yes	No	Yes
	$\bar{x}$ Residual			0.000	0.001	0.002
6.25%	N	23	N.D.	31	15	31
	$\bar{x}$	38.2		35.8	36.1	39.2
	S.D.	2.82		3.68	1.85	2.54
	SDDW	No		No	No	Yes
	SDND			Yes	No	No
	$\bar{x}$ Residual			0.000	0.000	0.000
12.50%	N	18	49	N.D.	29	N.D.
	$\bar{x}$	32.4	37.2		33.4	
	S.D.	1.81	1.80		1.86	
	SDDW	Yes	Yes		No	
	SDND		Yes		No	
	$\bar{x}$ Residual		0.001		0.001	
25.00%	N	22	9	21	15	N.D.
	$\bar{x}$	35.9	34.2	29.2	28.8	
	S.D.	1.85	2.06	1.58	0.98	
	SDDW	Yes	No	Yes	Yes	
	SDND		Yes	Yes	Yes	
	$\bar{x}$ Residual		0.021	0.000	0.000	
50.00%	N	N.D.	N.D.	N.D.	N.D.	N.D.
	$\bar{x}$					
	S.D.					
	SDDW					
	SDND					
	$\bar{x}$ Residual					
100.00%	N	N.D.	N.D.	N.D.	N.D.	N.D.
	$\bar{x}$					
	S.D.					
	SDDW					
	SDNS					
	$\bar{x}$ Residual					

[a] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in the same concentration in the nondisinfected effluent stream.

[b] Significantly different (P=0.05) by Dunnett's Test from the mean length of fish reared in dilution water tanks of the respective effluent stream.

[c] No data available.

of any of the other effluent streams tested. This trend was also seen when mean body weights at termination were compared. No fish survived to day 310 in the 100 percent concentration of any effluent stream, but the mean weights of fish in 50 percent ozonated effluent were at least 50 percent greater than the mean weights of fish in any other 50 percent effluent concentration. Due to mortality, the second generation data (Tables 27 and 28) contribute no information to this idea. However, in the Grandville study,[8] mean lengths of both first and second generation fish in 100 percent ozonated effluent, as well as mean weights of fish in the 100 percent ozonated effluent, were greater than the respective values for fish in comparable concentrations of any other effluent stream. Thus, the raw data from this project support the idea that fathead minnows grew better in very high concentrations of ozonated effluent than in comparable concentrations of the other effluent streams tested. Since growth was always greatest in intermediate effluent concentrations, it appeared that the effect of ozonation was to reduce the growth inhibiting characteristic(s) inherent in the effluent, rather than to directly promote superior growth. None of the measurements made in this study indicate what growth inhibiting factor(s) may have been surpressed by the ozonation process.

Reproduction

The effects of the various effluent streams on the reproductive functions of the fathead minnow were analyzed from the standpoint of egg production and egg hatchability. Egg production is defined as the number of viable eggs produced per female or per spawning in each concentration of each effluent stream. Hatchability refers to the percentage of eggs that hatched in incubation attempts in the various concentrations of the different effluent streams.

Tables 29 and 30 summarize the spawning activity in all effluent concentrations. The inherently toxic nature of high effluent concentrations manifested itself clearly with regard to egg production which was greatly reduced at concentrations higher than 6.25 - 12.5 percent effluent (Table 29). Poor survival of adult fish to reproductive age as a result of several outbreaks of an unidentified disease contributed to reduced and variable egg production in the low concentrations of nondisinfected effluent. All of the fish died prior to reproductive age in at least one of the duplicate tanks in all concentrations below 25 percent nondisinfected effluent. The fish that did produce eggs in all nondisinfected effluent concentrations except 6.25 percent deposited considerable fewer viable eggs per female than were observed for the same concentrations of nondisinfected effluent in the Grandville study[8] and for similar concentrations in the work of Arthur, et al.,[1] with nondisinfected effluent. The source of raw wastewaters in the latter two studies was almost entirely domestic.

The fact that essentially no egg production was observed in the 25 and 50 percent effluent concentrations even though fish survived those exposures indicates that the poor quality trickling filter effluent from wastewater with 35-45 percent industrial input severely inhibited the reproductive activities of the fathead minnow. This inherent inhibitory effect of the effluent, as well as the erratic impact of the unidentified disease upon a

Table 29. MEAN NUMBER OF VIABLE EGGS PRODUCED PER FEMALE AND THE MEAN CHEMICAL RESIDUAL (mg/l) IN EACH CONCENTRATION OF EACH EFFLUENT STREAM

Effluent Stream	Nominal percent effluent concentrations								
	0.000	1.56	3.12	6.25	12.50	18.75	25.00	50.00	100.00
Nondisinfected									
x̄ Egg/female	195[a]	0[c]	0[a]	569[a]	372[a]	b	2	0	0
Chlorinated									
x̄ Eggs/female	386	1262	46	2	0	21	2	0[c]	b
x̄ Residual	0.002	0.009	0.012	0.018	0.040	0.059	0.085	0.299	
Dechlorinated									
x̄ Eggs/female	1049	437	1343	922	0[a]	b	0	0	0[c]
x̄ Residual	0.000	0.000	0.000	0.000	0.001		0.005	0.048	0.010
Chlorobrominated									
x̄ Eggs/female	1211	1176[a]	1059[a]	126	1[a]	b	8	0	0[c]
x̄ Residual	0.003	0.005	0.005	0.007	0.006		0.005	0.008	0.009
Ozonated									
x̄ Eggs/female	333	672	1383	462	336	b	18	31	0[c]
x̄ Residual	0.001	0.002	0.002	0.002	0.002		0.002	0.002	0.003

[a] One of the two duplicate tanks had zero survivors at termination of the study

[b] No equivalent effluent concentration

[c] All fish in this concentration died prior to reproductive age

Table 30. MEAN NUMBER OF EGGS PER SPAWNING IN THE VARIOUS CONCENTRATIONS OF EACH EFFLUENT STREAM

Effluent Stream	Nominal percent effluent concentrations								
	0.00	1.56	3.12	6.25	12.50	18.75	25.00	50.00	100.00
Nondisinfected									
$\bar{x}$ Eggs/spawning	151[a]	130[b]	11[a]	219[a]	197[a]	c	86		b
No. of spawnings	23	17	2	49	13		4	0	0
Chlorinated									
$\bar{x}$ Eggs/spawning	130	190	94	16		90	36	b	c
No. of spawnings	72	81	7	3	0	4	1	0	
Dechlorinated									
$\bar{x}$ Eggs/spawning	163	183	231	187	111[a]	c			b
No. of spawnings	110	41	105	139	1		0	0	0
Chlorobrominated									
$\bar{x}$ Eggs/spawning	279	190[a]	190[a]	124	41[a]	c	197		b
No. of spawnings	24	100	46	23	1		2	0	0
Ozonated									
$\bar{x}$ Eggs/spawning	116	148	180	170	134	c	72	157	b
No. of spawnings	60	78	196	29	38		3	2	0

[a] One of the two duplicate tanks had zero survivors at termination of the study

[b] Both duplicate tanks had zero survivors at termination of the study

[c] No equivalent effluent concentration

number of test tanks in different effluent streams, made it impossible to assess the effect of any chemical treatment upon egg production.

Some of the viable eggs produced were incubated to determine their hatchability (i.e., the percent of eggs per spawning that hatched and produced fry that were living at the end of the five-day incubation period). Fry that had hatched, or partially hatched, but were not alive at the end of the incubation period were not considered living fry.

A summary of the results of tests with eggs spawned and incubated in the same effluent type and concentration are shown in Table 31. This table is incomplete because viable egg production was limited to only the lower effluent concentrations. However, Table 31 does show that, with but one exception (6.25 percent ozonated effluent), the highest percent egg hatchability on each treatment table occurred in 100 percent dilution water. Addition to the dilution water of the smallest amount of effluent, whether it was disinfected or not, inhibited egg hatchability. Thus, the reduced hatchability recorded in this table appeared to result from characteristics of the raw effluent rather than the chemical treatment applied to any effluent stream.

The deleterious nature of the raw effluent was further documented when eggs spawned in dilution water were incubated in the higher concentrations of the various effluent streams (Table 32). No eggs hatched in the 12.5-100 percent nondisinfected effluent concentrations and no significant hatchability was observed in any disinfected effluent stream at concentrations greater than 12.5 percent.

In summary, the observations on the reproductive activity of the test subjects in this study illustrated the harmful effects of the nondisinfected effluent produced by the Wyoming plant, but did not identify any deleterious effects attributable to the chemical treatments applied to the various test streams.

Table 31. PERCENT HATCHABILITY, MEAN CHEMICAL RESIDUAL (mg/l), AND INCUBATION ATTEMPTS IN THE VARIOUS EFFLUENT STREAMS

	Nominal Percent Effluent Concentration								
Effluent Type	0.00	1.56	3.12	6.25	12.50	18.75	25.00	50.00	100.00
Nondisinfected									
% Hatchability	68	45		57	0	a			
No. of incubations	4	4	0	11	5		0	0	0
Chlorinated									
% Hatchability	82	76	46						
No. of incubations	19	28	2	0	0	0	0	0	a
Mean Residual	0.000	0.002	0.002	0.004	0.005	0.005	0.009	0.000	
Dechlorinated									
% Hatchability	86	73	78	61					
No. of incubations	38	9	25	24	0	a	0	0	0
Mean Residual	0.000	0.000	0.000	0.000	0.002		0.006	0.087	0.009
Chlorobrominated									
% Hatchability	89	75	69	76		a	10		
No. of incubations	12	25	11	2	0		1	0	0
Mean Residual	0.001	0.002	0.004	0.002	0.003		0.002	0.003	0.000
Ozonated									
% Hatchability	86	81	81	93	65	a		48	
No. of incubations	19	13	38	5	8		0	1	0
Mean Residual	0.000	0.001	0.001	0.001	0.001		0.001	0.001	0.001

[a] No equivalent effluent concentration

Table 32. PERCENT HATCHABILITY OF EGGS INCUBATED IN EFFLUENT BUT SPAWNED IN DILUTION WATER

Effluent Type	Test Group 1	Test Group 2	Test Group 3	Test Group 4
Nondisinfected				
Incubated in:	100% effluent	50% effluent	25% effluent	12.5% effluent
% Hatchability:	0	0	0	0
No. of attempts:	6	4	2	1
Chlorinated				
Incubated in:	50% effluent	25% effluent	18.75% effluent	
% Hatchability:	0	0	8.4	
No. of attempts:	4	4	5	
Dechlorinated				
Incubated in:	100% effluent	50% effluent	25% effluent	12.5% effluent
% Hatchability	0	0	0.7	24
No. of attempts:	20	17	3	2
Chlorobrominated				
Incubated in:	100% effluent	50% effluent	25% effluent	12.5% effluent
% Hatchability:	0	4.7	2.6	77.6
No. of attempts:	16	11	9	5
Ozonated				
Incubated in:	100% effluent	50% effluent		
% Hatchability:	0	0		
No. of attempts:	6	1		

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SECTION VI

ACUTE TOXICITY TESTS

INTRODUCTION

Acute toxicity tests are used to determine the amount of toxicant necessary to produce a given response (usually death) by a biological indicator population in a relatively short (normally 96 hours or less) period of time. While some species of fish and invertebrates do not lend themselves well to life cycle toxicity testing, almost any species that can be captured and maintained in the laboratory can be tested in acute toxicity tests. Results are often expressed as the TL50 or LC50, which is that level of toxicant required to kill half of the test animals during the exposure time under a given set of experimental conditions. The results of acute toxicity tests, along with those from life cycle tests, are useful in predicting the potential impact of toxicants upon aquatic life.

MATERIALS AND METHODS

Acute toxicity tests of 48-96 hours in duration were conducted using a variety of cold and warm water fishes. In addition, a number of species of aquatic insect larvae and several species of microinvertebrates were used in 48-hour tests. Materials and methods were identical to those used in the Grandville study,[1] except for the differences noted below.

In test chambers where low dissolved oxygen levels were potentially detrimental, oil-free air was introduced for aeration. The dissolved oxygen concentrations in all test aquaria were usually measured at 0, 48, and 96 hours into each test period. Due to industrial components in the effluent, occasional flows of chemicals of an unknown nature entered the effluent stream. When possible, chemical analyses were performed to identify the unknown substances.

The first three tests with fathead minnows using dechlorinated effluent, as well as the fathead minnow test with chlorinated effluent, were conducted using diluters calibrated to deliver to the duplicate test tanks 100 percent effluent, 100 percent dilution water, and six intermediate concentrations, each diluted 50 percent more than the immediately preceding higher concentration. For the remaining tests, the diluters were recalibrated to deliver 100 percent effluent, 100 percent dilution water, and six intermediate test concentrations, each receiving a volume of effluent equal to 60 percent of the immediately preceding higher concentration. This calibration change was made to minimize the difference in effluent concentration between those levels producing 100 percent mortality and those resulting in 100 percent survival of test animals. Ideally, effluent concentrations would be maintained at levels giving nearly 50 percent mortality. However, under actual experimental conditions, the Wyoming effluent quality was so variable that maintenance of a constant disinfectant residual level was virtually impossible.

Fish used in acute tests were either reared in the laboratory, purchased from private sources, collected from local streams, or obtained from State of Michigan or national fish hatcheries. Invertebrates were captured from local lakes and streams, or obtained in pure culture from the Environmental Research Laboratory - Duluth. Test animals were held under experimental temperature and light conditions for at least seven days prior to testing. During the acclimation period, fish were observed for signs of disease or parasites. Fish exhibiting symptoms of bacterial infection were treated with neomycin, tetracycline, terramycin, nitrofurazone, or furanace, while those showing evidence of ectoparasitic diseases were treated with formalin and/or malachite green. Total lengths (±0.5 mm) and weights (±0.1 g) were recorded for each fish that died during the test period.

Acute toxicity tests with invertebrates were started between 9 A.M. and 2 P.M. The insect larvae and other large invertebrates were transferred to the test tanks by hand. Rocks were provided in the test chambers to serve as shelter for those animals. The smaller invertebrates (copepods, cladocera, ostracods, and midge larvae) were captured and transported to the test chamber using small pipettes. These subjects were exposed to effluent in test containers which were specifically designed for testing small invertebrates. A 160 mm length of 16 mm O.D. glass tubing was immersed approximately 80 mm into the desired effluent concentration. The immersed end of the tubing was fitted with a fine mesh (34 strands/cm) stainless steel cap to prevent escape of the invertebrates. This glass cylinder was suspended from an apparatus which caused it to move vertically 18 mm through the water column in an up and down motion eight times per minute. Since the tanks in which the test containers were immersed were being continuously supplied by a flow-through diluter system, the invertebrates were exposed to the desired test concentration under flow-through conditions. This method also facilitated recapture for mortality determinations. Partial plugging of the stainless steel screening was a frequent problem in high effluent concentrations. The small size of the microinvertebrates made intermittent mortality and behavior observations impractical. Therefore, the TL50 was calculated based upon mortality counts made when the test was terminated. Invertebrates were observed under a dissecting microscope and were considered dead when they gave no response to gentle prodding. Ten animals were used per test chamber when possible, but when numbers of subjects were limited, as few as five were used per test container.

Most acute toxicity experiments were run during the life cycle test period with the same effluent used in the life cycle tests. However, after completion of the life cycle study, additional toxicity tests with O_3 were performed so that a TL50 could be calculated. To accomplish this objective, exceptionally high O_3 residuals were needed. Test aquaria were situated immediately adjacent to the O_3 contactor and were fed ozonated effluent direct from the contactor.

ACUTE TOXICITY TESTS WITH NONDISINFECTED EFFLUENT

As early as 1933[2] Hubbs expressed concern over man's failure to resolve the problem of reducing the harmful effects of wastewater effluent on fish life. He discussed the lack of legal limits of dissolved oxygen levels and biochemical oxygen demand, the harmful effects on fishing, recreation, health, and economics, and the potentially beneficial effects of proper sludge handling as topics that required more attention. He cited several instances of fish kills that could be attributed to wastewater effluent (nondisinfected) and reported that the state of New York was conducting bioassay studies to determine if there had been a violation of the law prohibiting discharges injurious to fish life.

Alabaster[3] studied a channel containing undiluted, nondisinfected wastewater effluent. The wastewater channel was connected to a relatively unpolluted canal and fish could move freely between the canal and the channel. Alabaster found coarse fish through the length of the wastewater channel, but fishing success and concentrations of dissolved oxygen were highest upstream from the discharge site and more than 2.5 miles downstream from the discharge site. Mortalities were often observed when caged fish were placed in the channel within one mile downstream from the point of discharge, although no comparable mortalities of free-living fish were ever observed. Alabaster concluded that the potential for an aquatic environment to support fish populations varied directly with dissolved oxygen levels, and that free-living fish avoided areas where mean dissolved oxygen levels were less than 1-2 mg/l as the result of effluent discharge.

Esvelt, et al.,[4] found that primary effluent, derived either from domestic or industrial wastewater sources, was toxic to golden shiners. The TL50's ranged from 39 to 63 percent effluent. They further found that the toxicity was effectively removed by activated sludge treatment and, to a lesser extent, by physical-chemical treatment, with acute test results ranging from 100 percent survival in undiluted effluent to 50 percent survival in 76 percent effluent.

In a study evaluating the acute toxicity of nondisinfected municipal wastewater effluent to several fishes and invertebrates, Arthur, et al.,[5] observed mortalities in excess of 50 percent of the test population in 2 of 12 tests, and greater than 20 percent in 6 of 12 tests. They concluded that the lethal responses were primarily the result of reduced dissolved oxygen levels.

Gaufin[6] found that heavy organic enrichment from an effluent discharge limited the distribution and species composition of the macroinvertebrate community in an Ohio trout stream. However, he also noted that approximately 11 miles downstream from the effluent discharge the stream had recovered completely and displayed a varied and well-balanced aquatic community.

Acute toxicity tests with nondisinfected effluent from the Wyoming plant resulted in mortality of eight of the nine fish species tested and one of the seven invertebrate species tested, particularly in the higher effluent concentrations (Table 33). The toxic nature of the nondisinfected trickling filter effluent was likely due to the extremely poor quality with respect to soluble and insoluble organic constituents (see Section III) and the unknown industrial components present in the source wastewater. Even though test tanks were cleaned daily, fish tested in 100 percent nondisinfected effluent were usually not visible due to the extremely turbid nature of the effluent. It was periodically necessary to pass a net through the test tank to determine if the test specimens had died.

The toxic nature of the effluent prior to the addition of any disinfectant is a factor worthy of consideration when reviewing the results of acute tests with disinfected effluents. In the cases of acute tests with dechlorinated and ozonated effluents, the mortality observed was usually at a level comparable to that experienced by the same species in nondisinfected effluent. This information, coupled with our previous findings, suggests that probably the effluent rather than the chemical treatment was the toxic factor in those situations.

ACUTE TOXICITY TESTS WITH CHLORINATED EFFLUENT

The chlorinated effluent was more toxic to fishes and invertebrates than any other effluent tested. The signs of stress typically evidenced by fish exposed to chlorinated effluent included gasping at the water surface, rapid gill movements, loss of equilibrium and erratic swimming movements, loss of slime coat, hemorrhaging at the gills and base of fins, and passive floating or resting on the bottom immediately prior to death. Mortality of *Daphnia* and copepods occurred in effluent concentrations as low as 4.7 percent. However, a few species of macroinvertebrates survived well in effluent with mean residual Cl_2 levels as high as 1.491 mg/l. Arthur, *et al.*,[5] also found macroinvertebrates to be less sensitive to Cl_2 than fish, although sufficient mortalities occurred in most of his tests to enable calculation of a seven-day TL50. The greater toxicity observed in our tests as compared to Arthur's may be the result of the greater hardness of our dilution water. Hardness might affect the toxicity of Cl_2 as it does other materials.[7]

Calculated TL50 values for fish in chlorinated effluent (Table 34) ranged from 0.029 mg/l for the pugnose shiner to 0.519 mg/l for goldfish in cold water. However, the pugnose shiner TL50 concentration of 0.029 mg/l Cl_2 is probably unrealistically low, since more than 50 percent mortality occurred in the 100 percent nondisinfected effluent concentration (Table 33). Most of the other values reported in Table 34 are similar to the TL50 values obtained in the Grandville study.[1] In most cases, the TL50 expressed in terms of percent chlorinated effluent was 35 to 50 percent of the TL50 when the same species was tested in nondisinfected effluent. This indicated that most of the acute toxicity tests were measuring toxicity associated with the disinfection process of chlorination and not merely the inherent toxicity of nondisinfected effluent.

Table 33. RESULTS OF ACUTE TOXICITY TESTS WITH NONDISINFECTED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 % Effluent	Inclusive Dates	Comments[a]
Fathead Minnow #1 *Pimephales promelas*	25		1-27 to 1-30-75	72 hour acute test; zero mortality in 100% effluent
Fathead Minnow #2 *Pimephales promelas*	25		2-11 to 2-12-75	24 hour acute test; zero mortality in 100% effluent
Fathead Minnow #3 *Pimephales promelas*	25		2-17 to 2-21-75	Zero mortality in 100% effluent
Northern Common Shiner *Notropis cornutus*	25	75	3-24 to 3-28-75	95% mortality in 100% effluent
Pugnose Shiner *Notropis anogenus*	25	65	5-12 to 5-16-75	100% mortality in 100% effluent
Rainbow Trout *Salmo gairdnerii*	15	55	3-18 to 3-21-75	72 hour acute test; 60% mortality in 60% effluent
Brown Trout *Salmo trutta*	14	55	3-18 to 3-21-75	72 hour acute test; 60% mortality in 60% effluent
Splake *Salvelinus sp.*	15	81	4-28 to 5-2-75	85% mortality in 100% effluent
Brook Trout *Salvelinus fontinalis*	15	77	4-28 to 5-1-75	100% mortality in 100% effluent
Lake Trout *Salvelinus namaycush*	15	38	5-4 to 5-8-76	100% mortality in 60% effluent

(continued)

[a] Percent mortality is given for the lowest concentration which had greater than 50 percent mortality if a TL50 was obtained; otherwise, percent mortality is given for the 100 percent effluent concentration.

Table 33 (continued). RESULTS OF ACUTE TOXICITY TESTS WITH NONDISINFECTED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 (% Effluent)	Inclusive Dates	Comments
Chinook Salmon *Oncorhynchus tshawytscha*	15	54	3-18 to 3-21-75	72 hour acute test; 65% mortality in 60% effluent
Mayfly larvae *Hexagenia Sp*.	16	18	5-8 to 5-10-75	60% mortality in 13% effluent
Mayfly larvae *Stenonema sp*.	18		8-28 to 8-30-75	30% mortality in 100% effluent
Caddisfly larvae Hydropsychidae	17		7-23 to 7-25-75	15% mortality in 100% effluent
Caddisfly larvae Limnephilidae	18		8-28 to 8-30-75	10% mortality in 100% effluent
Stonefly larvae Plecoptera	18		7-28 to 7-30-75	24 hour acute test; 10% mortality in 100% effluent
Isopods *Ascellus sp*.	17		7-23 to 7-25-75	15% mortality in 100% effluent
Cyclopoid copepods	18		12-9 to 12-11-75	10% mortality in 100% effluent

Table 34. RESULTS OF ACUTE TOXICITY TESTS WITH CHLORINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50[b] mg/1 Cl_2 (% effluent)	Inclusive Dates	Comments[a]
Fathead Minnow *Pimephales promelas*	25	0.120 (18%)	2-4 to 2-8-74	100% mortality at 0.300 mg/1 (25% effluent) $\bar{x}$ length 17 mm, $\bar{x}$ weight 0.1 g
Pugnose Shiner *Notropis anogenus*	26	0.029 (75%)	4-21 to 4-25-75	100% mortality at 0.029 mg/1 (100% effluent) $\bar{x}$ length 65 mm, $\bar{x}$ weight 3.1 g
Pugnose Minnow *Opsopoeodus emiliae*	25	0.085 (75%)	5-5 to 5-9-75	100% mortality at 0.104 mg/1 (100% effluent) $\bar{x}$ length 48 mm, $\bar{x}$ weight 1.0 g
Goldfish *Carassius auratus*	13	0.519 (55%)	3-9 to 3-11-76	60% mortality at 0.586 mg/1 (60% effluent) Test terminated at 48 hours $\bar{x}$ length 59 mm, $\bar{x}$ weight 2.6 g
Goldfish *Carassius auratus*	25		5-5 to 5-9-76	20% mortality at 0.071 mg/1 (100% effluent)
Brook Trout *Salvelinus fontinalis*	16	0.068 (33%)	4-14 to 4-18-75	60% mortality at 0.075 mg/1 (36% effluent) $\bar{x}$ length 42 mm, $\bar{x}$ weight 0.6 g
Rainbow Trout *Salmo gairdnerii*	16	0.037 (19%)	4-14 to 4-18-75	55% mortality at 0.041 mg/1 (22% effluent) $\bar{x}$ length 53 mm, $\bar{x}$ weight 1.5 g
Splake *Salvelinus sp.*	15	0.071 (30%)	4-14 to 4-18-75	80 % mortality at 0.095 mg/1 (36% effluent) $\bar{x}$ length 53 mm, $\bar{x}$ weight 1.1 g

(continued)

[a] Percent mortality is given for the lowest effluent concentration having greater than 50% mortality if a TL50 is obtained; otherwise, percent mortality is given for the 100% effluent concentration.

[b] Figures in parentheses are TL50 values calculated in terms of percent effluent

Table 34 (continued). RESULTS OF ACUTE TOXICITY TESTS WITH CHLORINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 mg/1 Cl_2 (% effluent)	Inclusive Dates	Comments
Chinook Salmon *Oncorhynchus tshawytscha*	15	0.065 (28%)	4-14 to 4-18-75	100% mortality at 0.095 mg/1 (36% effluent) $\bar{x}$ length 59 mm, $\bar{x}$ weight 1.8 g
Mayfly larvae *Hexagenia sp.*	16	0.357 (51%)	5-5 to 5-7-75	70% mortality at 0.400 mg/1 (60% effluent)
Caddisfly larvae Family Hydropsychidae	17		7-21 to 7-23-75	30% mortality at 1.361 mg/1 (100% effluent)
Caddisfly larvae Family Limnephilidae	17		9-8 to 9-10-75	Zero mortality at 1.491 mg/1 (100% effluent)
Stonefly larvae Order Plecptera	16	0.781 (60%)	8-6 to 8-8-75	60% mortality at 1.411 mg/1 (100% effluent)
Isopods *Ascellus sp.*	17		7-16 to 7-18-75	25% mortality at 1.624 mg/1 (100% effluent)
Amphipod *Grammarus sp.*	17	1.102 (77%)	7-21 to 7-23-75	100% mortality at 1.361 mg/1 (100% effluent)
Snails *Goniobasis livescens*	16		8-6 to 8-8-75	Zero mortality at 1.411 mg/1 (100% effluent). Snails exposed to high concentrations of chlorinated effluent withdrew into their shells, but emerged again when placed in well water upon completion of the test.
Daphnia magna	16	0.045 (4%)	10-14 to 10-16-75	100% mortality at 0.054 mg/1 (4.7% effluent)
Calanoid copepod *Epischura lacustris*	14	0.063 (5%)	9-17 to 9-19-75	100% mortality at 0.086 mg/1 (7.8% effluent)
Assorted Calanoid and Cyclopoid copepods	14	0.041 (4%)	10-8 to 10-10-75	67% mortality at 0.044 mg/1 (4.7% effluent)

ACUTE TOXICITY TESTS WITH DECHLORINATED EFFLUENT

As in the Grandville study,[1] the mortality pattern of fish exposed to dechlorinated effluent was similar to the pattern in nondisinfected effluent (Table 35). It appears that the inherent toxicity of the effluent was the likely cause of the mortality rather than residual sulfite.

All invertebrates exposed to dechlorinated effluent exhibited a response similar to those tested in nondisinfected effluent, which again indicated the high acute tolerance of many invertebrates to wastewater effluent. In fact, in most cases, there was slightly more mortality when invertebrates were exposed to nondisinfected effluent as compared to those tested in dechlorinated effluent. Due to aeration of all concentrations, low dissolved oxygen levels did not appear to have been a factor affecting the results of either the dechlorinated or nondisinfected acute tests (the mean dissolved oxygen level of all 100 percent nondisinfected acute tanks was 6.0 mg/l, while the mean dissolved oxygen concentration of all 100 percent dechlorinated acute test chambers was 6.5 mg/l).

ACUTE TOXICITY TESTS WITH CHLOROBROMINATED EFFLUENT

Most acute test fish in chlorobrominated effluent exhibited mortality patterns and stress symptoms similar to those tested in chlorinated effluent (Table 36). An exception was the salmonid fishes which proved to be more sensitive to chlorobrominated effluent than to chlorinated effluent. Less than half of the amount of residual BrCl was necessary to produce a TL50 when compared to similar tests run with chlorinated effluent, although TL50 values for chlorobrominated effluent expressed as percent effluent were slightly higher than those for chlorinated effluent. These results are in contrast with our Grandville findings with chlorobrominated activated sludge effluent,[1] in which we found chlorobrominated effluent to be less toxic than chlorinated effluent. These results also disagree with Zillich's[8] conclusion that chlorobrominated effluent was no more toxic than nondisinfected effluent. On the other hand, little mortality was recorded in chlorobrominated acute tests with macroinvertebrates, while several species were killed in sufficient numbers to calculate a TL50 when exposed to chlorinated effluent.

The apparent difference between the toxic effects of chlorobrominated effluent at Grandville and at Wyoming might have been the result of an additive or synergistic interaction involving BrCl and some other component of the effluent. On one occasion (and probably others, although comprehensive observations were available for only the one reported incident), an apparently synergistic reaction involving BrCl and cyanide occurred. Seven hours after the beginning of an acute toxicity experiment involving exposure of fathead minnows to chlorobrominated effluent, no mortalities or unusual responses had yet been observed. However, the next morning, after 22 hours of exposure, total mortality had occurred in the 100, 50 and 25 percent effluent tanks, and 90 percent mortality in the 12.5 percent effluent tanks. Ampereometric measurement of BrCl at that time indicated only 0.127-0.015 mg/l residual BrCl in the 100-12.5 percent effluent tanks, although the titrations were erratic and unstable. Immediately after the

Table 35. RESULTS OF ACUTE TOXICITY TESTS WITH DECHLORINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50[b] mg/1 SO_2 (% effluent)	Inclusive Dates	Comments[a]
Fathead Minnow #1 *Pimephales promelas*	25		10-15 to 10-19-73	20% mortality at 0.001 mg/1 (100% effluent) $\bar{x}$ length 21 mm, $\bar{x}$ weight 0.2 g
Fathead Minnow #2 *Pimephales promelas*	25		10-22 to 10-26-73	Zero mortality at 0.000 mg/1 (100% effluent)
Fathead Minnow #3 *Pimephales promelas*	25	0.273 (74%)	2-4 to 2-8-74	100% mortality at 0.428 mg/1 (100% effluent) $\bar{x}$ length 18 mm, $\bar{x}$ weight 0.2 g. The dissolved oxygen level in the 100% effluent tanks was less than 1.0 mg/1 when the fish were found dead. In this case, mortality was probably the result of anoxia rather than residual sulfur dioxide.
Fathead Minnow #4 *Pimephales promelas*	25		1-27 to 1-30-75	5% mortality in 100% effluent $\bar{x}$ SO_2 residual 2.64 mg/1
Northern Common Shiner *Notropis cornutus*	15	0.117 (100%)	4-14 to 4-18-75	50% mortality at 0.117 mg/1 (100% effluent) $\bar{x}$ length 55 mm, $\bar{x}$ weight 1.5 g
Brook Trout *Salvelinus fontinalis*	14	1.400 (77%)	4-7 to 4-11-75	100% mortality at 3.52 mg/1 (100% effluent) $\bar{x}$ length 40 mm, $\bar{x}$ weight 0.6 g
Brown Trout *Salmo trutta*	14	0.117 (42%)	4-7 to 4-11-75	95% mortality at 0.142 mg/1 (60% effluent) $\bar{x}$ length 45 mm, $\bar{x}$ weight 1.0 g

(continued)

[a] Percent mortality is given for the lowest effluent concentration having greater than 50% mortality if a TL50 is obtained; otherwise, percent mortality is given for the 100% effluent concentration.

[b] Figures in parentheses are TL50 values calculated in terms of percent effluent for comparison purposes.

Table 35 (continued). RESULTS OF ACUTE TOXICITY TESTS WITH DECHLORINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 mg/1 SO_2 (% effluent)	Inclusive Dates	Comments
Rainbow Trout *Salmo gairdnerii*	16	1.185 (82%)	5-12 to 5-16-75	80% mortality at 1.894 mg/1 (100% effluent) $\bar{x}$ length 64 mm, $\bar{x}$ weight 2.9 g
Lake Trout *Salvelinus namaycush*	16	0.325 (77%)	5-10 to 5-11-76	100% mortality at 0.488 mg/1 (100% effluent) $\bar{x}$ length 56 mm, $\bar{x}$ weight 1.1 g
Splake *Salvelinus sp.*	14	1.670 (81%)	4-7 to 4-11-75	85% mortality at 3.52 mg/1 (100% effluent) $\bar{x}$ length 47 mm, $\bar{x}$ weight 0.8 g
Chinook Salmon *Oncorhynchus tshawytscha*	14	0.190 (63%)	4-7 tp 4-11-75	100% mortality at 3.11 mg/1 (100% effluent) $\bar{x}$ length 57 mm, $\bar{x}$ weight 1.8 g
Stonefly larvae Order Plecoptera	18		7-30 to 8-1-75	Zero mortality at 0.000 mg/1 (100% effluent)
Caddisfly larvae Family Hydropsychidae	17		8-11 to 8-13-75	Zero mortality at 0.000 mg/1 (100% effluent)
Amphipods *Gammarus sp.*	18		7-30 to 8-1-75	Zero mortality at 0.000 mg/1 (100% effluent)
Isopods *Ascellus sp.*	17		8-11 to 8-13-75	Zero mortality at 0.000 mg/1 (100% effluent)
Snails *Goniobasis livescens*	17		8-11 to 8-13-75	Zero mortality at 0.000 mg/1 (100% effluent)
Cyclopoid copepods	17		12-1 to 12-3-75	10% mortality at 0.034 mg/1 (100% effluent)

Table 36. RESULTS OF ACUTE TOXICITY TESTS WITH CHLOROBROMINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50[b] mg/1 BrCl (% effluent)	Inclusive Dates	Comments[a]
Fathead Minnow Pimephales promelas	25		1-28 to 1-29-75	See text
Fathead Minnow Pimephales promelas	25		2-18 to 2-21-75	72 hour acute test Zero mortality at 0.143 mg/1 (100% effluent)
Pugnose Shiner Notropis anogenus	25	0.010 (55%)	4-28 to 5-2-75	60% mortality at 0.010 mg/1 (60% effluent) $\bar{x}$ length 59 mm, $\bar{x}$ weight 2.0 g
Goldfish Carassius auratus	15		4-26 to 4-28-76	Zero mortality at 0.000 mg/1 (100% effluent). Test terminated after 54 hours.
Rainbow Trout Salmo gairdnerii	14	0.017 (28%)	4-21 to 4-25-75	100% mortality at 0.020 mg/1 (36% effluent) $\bar{x}$ length 56 mm, $\bar{x}$ weight 2.0 g
Brook Trout Salvelinus fontinalis	14	0.020 (44%)	4-21 to 4-25-75	100% mortality at 0.019 mg/1 (60% effluent)
Splake Salvelinus sp.	15	0.019 (35%)	4-21 to 4-25-75	55% mortality at 0.019 mg/1 (36% effluent) $\bar{x}$ length 53 mm, $\bar{x}$ weight 0.9 g
Lake Trout Salvelinus namaycush	15	46 Percent Chlorobrominated effluent	4-26 to 4-28-76	100% mortality at 0.002 mg/1 (60% effluent). Insufficient amounts of bromine chloride were measured for determination of a TL50 expressed as mg/1 BrCl.
Chinook Salmon Oncorhynchus tshawytscha	15	0.016 (28%)	4-21 to 4-25-75	100% mortality at 0.019 mg/1 (36% effluent) $\bar{x}$ length 64 mm, $\bar{x}$ weight 2.4 g

(continued)

[a] Percent mortality is given for the lowest effluent concentration having greater than 50% mortality if a TL50 is obtained; otherwise, percent mortality is given for the 100% effluent concentration.

[b] Figures in parentheses are TL50 values calculated in terms of percent effluent.

Table 36 (continued). RESULTS OF ACUTE TOXICITY TESTS WITH CHLOROBROMINATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 mg/1 BrCl (% effluent)	Inclusive Dates	Comments
Mayfly larvae *Stenonema sp.*	16		8-25 to 8-27-75	10% mortality at 0.032 mg/1 (100% effluent)
Caddisfly larvae Family Limnephilidae	16		8-25 to 8-27-75	Zero mortality at 0.032 mg/1 (100% effluent)
Stonefly larvae Order Plecoptera	17		8-20 to 8-22-75	Zero mortality at 0.035 mg/1 (100% effluent)
Snails *Goniobasis livescens*	17		8-20 to 8-22-75	Zero mortality at 0.034 mg/1 (100% effluent). Snails exposed to high concentrations of chlorobrominated effluent withdrew into their shells, but emerged again when placed in well water.
Midge *Tanytarsus dissimilis*	17		11-17 to 11-19-75	Zero mortality at 0.037 mg/1 (100% effluent)
Ostracods Subclass ostracoda	17		11-17 to 11-19-75	20% mortality at 0.037 mg/1 (100% effluent)
Calanoid copepods	17	0.002 (4%)	10-21 to 10-23-75	100% mortality at 0.006 mg/1 (7.8% effluent)
Cyclopoid copepods	17		11-11 to 11-13-75	10% mortality at 0.030 mg/1 (36% effluent)
Cladoceran *Simocephalus serrulatus*	17	0.023 (47%)	11-13 to 11-15-75	60% mortality at 0.026 mg/1 (60% effluent)

dead fish were removed, ten more fathead minnows were placed in the 100 and 50 percent chlorobrominated effluent tanks. All of the fish died within 27 minutes of exposure in the 100 percent effluent. There was 90 percent mortality in the 50 percent effluent tank after 6.5 hours of exposure. Moreover, fathead minnows exposed at the same time to the same concentrations of nondisinfected and dechlorinated effluents were not adversely affected. The BrCl residual level in the 100 percent tank at that time was 0.127 mg/l, and the mean residual level in the 50 percent tank was 0.101 mg/L Both of these figures are well below TL50 values obtained in earlier work. A cyanide analysis was performed at the time of the test mortalities. It was found that 1.10 mg/l total cyanide was present in the undiluted effluent. Perhaps the cyanide, or cyanide in combination with a metallic plating waste, combined with the BrCl to produce an extremely toxic environment for the fish. We observed this phenomenon on one other occasion during preliminary testing.

ACUTE TOXICITY TESTS WITH OZONATED EFFLUENT

Acute toxicity tests with ozonated effluent produced less mortality than tests with any other type of effluent (Table 37). However, the effluent was filtered prior to ozonation, and this may have been a significant factor contributing to the reduced toxicity observed. Acute toxicity of undiluted ozonated effluent to cladocera was the only routine test wherein sufficient mortalities occurred to enable calculations of a TL50. It is unlikely that the observed mortality was caused by the residual ozone, the concentration of which (0.001 mg/l) was at the threshold of detectability. These results are in agreement with previous tests[1] and with the findings of Arthur, et al.,[5] who observed in six different acute tests less mortality in 100 percent ozonated effluent than in 100 percent nondisinfected effluent. Evans[9] suggested that ozone has the advantages of removing taste, odor, and color, addition of oxygen, and reduction of BOD and COD, all of which may have contributed to the reduced toxicity observed in ozonated effluent. However, as early as 1933 Hubbs[2] noted that 0.10 mg/l of O_3 in water was sufficient to kill small fishes, and that smaller quantities, perhaps less than 0.01 mg/l, "sufficed to throw the fish in nervous movements, from which there was no quick recovery". Arthur, et al.,[5] also observed mortalities of fathead minnows exposed to O_3 residual levels of 0.20-0.30 mg/l.

Rosenlund[10] observed high mortalities when rainbow trout were exposed to ozonated Lake Mohave water. However, the O_3 residual at the head of his experimental tanks ranged from 0.01 to 0.06 mg/l, while the highest O_3 residual measured in any of our routine test tanks was 0.012 mg/l, with the majority of the observed residual levels being considerably lower (0.002 to 0.008 mg/l).

Some acute tests were conducted with activated sludge effluent receiving exceptionally high doses of O_3. Fish were exposed to the ozonated effluent immediately after O_3 addition. The results of these tests (Table 38) indicate that O_3, in concentrations ranging from 0.088 to 0.288 mg/l, is sufficiently toxic to cause mortality. Symptoms of stress evidenced by test fish included swimming at the surface, loss of equilibrium, and quiescent periods at the surface or bottom. Lake trout were least resistant, while

Table 37. RESULTS OF ACUTE TOXICITY TESTS WITH OZONATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 mg/1 O_3 (% effluent)	Inclusive Dates	Comments[a]
Fathead Minnow *Pimephales promelas*	16		5-20 to 5-21-76	[c]100% mortality at 0.225 mg/1 (100% effluent). All dead within 20 hours. $\bar{x}$ length 59 mm, $\bar{x}$ weight 2.3 g
Fathead Minnow *Pimephales promelas*	16		5-26 to 5-27-76	[c]Zero mortality at 0.059 mg/1 (100% effluent). Fish exposed for a total of 27 hours.
Northern Common Shiner *Notropis cornutus frontinalis*	25		4-7 to 4-11-75	40% mortality at 0.003 mg/1 (100% effluent)
Goldfish *Carassius auratus*	16		5-20 to 5-24-76	[c]100% mortality at 0.288 mg/1 (100% effluent) $\bar{x}$ survival time = 62.7 hours $\bar{x}$ length 77 mm, $\bar{x}$ weight 5.6 g
Brown Trout *Salmo trutta*	13		3-24 to 3-26-75	10% mortality at 0.008 mg/1 (100% effluent) This test was 48 hours in duration.
Splake *Salvelinus sp.*	13		3-24 to 3-26-75	5% mortality at 0.007 mg/1 (100% effluent) This test was 48 hours in duration.
Lake Trout *Salvelinus namaycush*	16		6-18-76	[c]100% mortality at 0.126 mg/1 (100% effluent) $\bar{x}$ survival time = 61 minutes $\bar{x}$ length 58 mm, $\bar{x}$ weight 1.4 g

(continued)

[a] Percent mortality is given for the lowest effluent concentration having greater than 50% mortality if a TL50 is obtained; otherwise, percent mortality is given for the 100% effluent concentration.

[b] Figures in parentheses are TL50 values calculated in terms of percent effluent.

[c] Fish were exposed to ozonated effluent immediately after the ozone was introduced.

Table 37.(continued). RESULTS OF ACUTE TOXICITY TESTS WITH OZONATED EFFLUENT

Species	Test Temp. (C)	96 Hour TL50 mg/1 O_3 (% effluent)	Inclusive	Comments
Lake Trout *Salvelinus namaycush*	16		5-19-76	[c]100% mortality at 0.234 mg/1 (100% effluent) $\bar{x}$ survival time = 42 minutes 100% mortality at 0.048 mg/1 (50% effluent) $\bar{x}$ survival time = 4.4 hours $\bar{x}$ length 61 mm, $\bar{x}$ weight 1.9 g
Lake Trout *Salvelinus namaycush*	16		5-25 to 5-26-76	[c]100% mortality at 0.088 mg/1 (100% effluent). All dead within 21 hours. $\bar{x}$ length 63 mm, $\bar{x}$ weight 2.0 g
Chinook Salmon *Oncorhynchus tshawytscha*	13		3-24 to 3-26-75	5% mortality at 0.008 mg/1 (100% effluent). This test was 48 hours in duration.
Stoneflies Order Plecoptera	18		8-4 to 8-6-75	Zero mortality at 0.005 mg/1 (100% effluent)
Caddisflies Family Hydropsychidae	18		8-13 to 8-15-75	30% mortality at 0.009 mg/1 (100% effluent)
Amphipod *Gammarus sp.*	18		8-4 to 8-6-75	Zero mortality at 0.005 mg/1 (100% effluent)
Isopods *Ascellus sp.*	18		8-13 to 8-15-75	10% mortality at 0.009 mg/1 (100% effluent)
Snail *Goniobasis livescens*	18		8-4 to 8-6-75	Zero mortality at 0.005 mg/1 (100% effluent)
Cyclopoid Copepod	18		11-20 to 11-22-75	Zero mortality at 0.001 mg/1 (100% effluent)
Cladoceran *Simocephalus serrulatus*	18	0.001 (100%)	11-24 to 11-26-75	50% mortality at 0.001 mg/1 (100% effluent)

Table 38. RESULTS OF ACUTE TOXICITY TESTS WITH OZONATED ACTIVATED SLUDGE EFFLUENT[a]

Species	Test Temp. (C)	Inclusive Dates	Comments
Fathead Minnow *Pimephales promelas*	16	5-20 to 5-21-76	100% mortality at 0.225 mg/1 (100% effluent). All dead within 20 hours. $\bar{x}$ length 59 mm, $\bar{x}$ weight 2.3 g
Fathead Minnow *Pimephales promelas*	16	5-26 to 5-27-76	Zero mortality at 0.059 mg/1 (100% effluent). Fish exposed for a total of 27 hours.
Goldfish *Carassius auratus*	16	5-20 to 5-24-76	100% mortality at 0.288 mg/1 (100% effluent). $\bar{x}$ survival time = 62.7 hours. $\bar{x}$ length 77 mm, $\bar{x}$ weight 5.6 g
Lake Trout *Salvelinus namaycush*	16	5-19-76	100% mortality at 0.234 mg/1 (100% effluent). $\bar{x}$ survival time = 42 minutes. 100% mortality at 0.048 mg/1 (50% effluent). $\bar{x}$ survival time = 4.4 hours. $\bar{x}$ length 61 mm, $\bar{x}$ weight 1.9 g
Lake Trout *Salvelinus namaycush*	16	5-25 to 5-26-76	100% mortality at 0.088 mg/1 (100% effluent). All dead within 21 hours. $\bar{x}$ length 63 mm, $\bar{x}$ weight 2.0 g
Lake Trout *Salvelinus namaycush*	16	6-18-76	100% mortality at 0.126 mg/1 (100% effluent). $\bar{x}$ survival time = 61 minutes. $\bar{x}$ length 58 mm, $\bar{x}$ weight 1.4 g

[a] The characteristics of the activated sludge effluent were 49 mg/1 BOD, 67 mg/1 total suspended solids, 0.9 mg/1 total phosphate, 7.9 mg/1 NH_3-N, and pH 7.5.

goldfish appeared most tolerant, which is the typical response pattern of fish species to halogens and other oxidants. However, it is unlikely that fish would encounter such high O_3 levels in a receiving stream. Ozone residuals dissipate rapidly, as evidenced in this project which reports that after only five minutes of contact time the residual level was commonly reduced by an order of magnitude.

SUMMARY

The results of our acute toxicity tests indicate that the nondisinfected effluent used in these studies was inherently toxic to most species of fish tested, with TL50 values ranging from 38-81 percent effluent. Invertebrates were generally more tolerant of nondisinfected effluent than were fishes. Ozonation coupled with filtration appeared to eliminate the toxicity associated with the effluent, although exposure of fish to ozonated activated sludge effluent immediately after O_3 addition caused mortality. Salmonid fishes were more sensitive to chlorobrominated effluent than to any other effluent type, while most invertebrates tested showed similar tolerance to chlorobrominated effluent as to nondisinfected effluent. Chlorinated effluent was the most toxic effluent type tested, with sufficient mortality occurring in all but four tests to calculate a TL50. TL50 values calculated in terms of percent chlorinated effluent were generally lower than for any other effluent stream. Dechlorination with sulfur dioxide reduced the toxicity of the chlorinated effluent to levels similar to nondisinfected effluent for both fish and invertebrates.

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